

nature

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Nobel Prizes should give encouragement

WITH this year's Nobel Prizes awarded, it may be appropriate now to ask whether there is new ground to be broken in the future by changes in criteria applied to selection for the prizes reflecting changes in the nature of scientific enquiry itself and its relationship to society. Certainly in recent years there has been a cautious and belated recognition of astronomy as a subject appropriate for the physics prize. But there still remain large areas of scientific endeavour yet to be included within the orbit of the prizes, of which the earth sciences is perhaps the most conspicuous example. Within the present terms of award of the prizes these areas are unlikely ever to be recognised by the prize committees.

Many would argue that the prizes ought in any case to be phased out as they represent an outdated reward system, generate a fair amount of bitterness and jealousy, and often convey a spurious air of authority by their award that is misinterpreted not just by the general public but even by the scientists themselves, who should know better. If the prizes are simply seen as the final often belated stamp of approval on a fine piece of work, then these arguments have considerable force. But they surely could be, and (with the exception of the peace prize) rarely are, a way of saying "this activity looks interesting. By awarding it a prize we will be giving it encouragement and will be drawing the attention of a much wider public to it."

It goes without saying that such an approach is much more risky. The work in question may end up in disappointment, and the publicity afforded to it may have been damaging. And much excellent work which at present leads ultimately to the award of a prize will fail to be recognised at the time. But it may well be that an aura of infallibility around the Nobel Prizes could be dispensed with.

As it happens, 1979 would be a particularly appropriate year for the prize committee to explore a new approach. Whatever the expected shortcomings of the United Nations Conference on Science and Technology for Development in Vienna next August, none but the most blinkered will by then be ignorant of the questions being raised about science in and for the developing world. One of the more frequently heard complaints by those involved in scientific work in the developing world is that the seductive attractions of a western-style academic science are too great for too many of the most highly intelligent, who abandon to less competent people the much more immediately relevant problems lacking reward-studded lustre. Nobel Prizes have been one of the ways of conferring this lustre. What could be more appropriate than that in 1979 a serious effort was made to identify work being done in the Third World of wide impact, even if not of exalted scholarship, and give to it an imaginative dose of encouragement. □

Victims of censorship

EVERY week roughly 20,000 copies of *Nature* go into the mail. Of these, rather more than 80% leave the United Kingdom for all parts of the globe from China to Peru. As far as we can tell they all reach their destination (barring the odd few that go astray), with one major exception. Certain countries in Eastern Europe take it upon themselves to censor the incoming mail of their citizens.

The pattern is not uniform; some countries don't bother, others are ruthless. By far the biggest offender is the Soviet Union. When there is any mention of that country in our news pages, whether it is favourable, neutral or critical, copies of that particular issue are

often simply thrown away at the border. As a result our Soviet readers see, at best, half of all issues of the journal.

There are ways to try to beat the system, of course, but they are only marginally effective and temporary expedients. What is needed, and what is most unlikely to occur, is for governments given to these extraordinary petty gestures to recognise first that all intelligent scientists in their country know perfectly well much of what they are trying to suppress, and second that one of the most effective ways of crippling the growth of science is to inhibit the free flow of scientific literature. □



Funding delays handicap ISABELLE in race for new particle

The US Department of Energy has extended construction time for Brookhaven's 400 × 400 GeV accelerator from five to seven years. **David Dickson** reports on the implications.

PHYSICISTS at the Brookhaven National Laboratory on New York's Long Island are trying to persuade the Department of Energy to reverse its decision to extend the construction period of its new 400 × 400 GeV intersecting storage accelerator (ISABELLE).

At stake is a race with the European Organisation for Nuclear Research (CERN) in Geneva to demonstrate the existence of the theoretically predicted intermediate vector boson, known as the W particle. According to the Weinberg-Salam unification theory, this particle carries the weak force in the same way that the photon carries the electromagnetic force.

Initially, Brookhaven's scientists hoped ISABELLE's larger number of interactions would compensate for CERN's planned earlier start in the search for the particle using proton-antiproton interactions. The extra two years' delay in construction could, it is feared, considerably reduce chances of being first past the post.

Construction work on what has been described as the largest basic research project ever built began last month. When completed, the 2½-mile circumference accelerator will provide a range of detection facilities for studying the results of collisions between two beams of opposite circulating protons.

The Department of Energy, under pressure from members of Congress, agreed last year to put \$23 million to begin construction work on ISABELLE into its budget request for 1979; when this money officially became available last month, a ground-breaking ceremony took place.

However, in an attempt to keep the lid on funding for high energy physics over the next few years, the department later announced that the accelerator's construction schedule would be increased from five years to seven. The first experiments have been pushed back from 1984 to 1986.

ISABELLE has been designed to make use of pulses of 30 GeV protons from the Alternating Gradient Synchrotron, Brookhaven's last major high energy physics facility which came into operation in 1961. The pulses will be stacked sequentially in each ring until 10 amps of circulating current has been built up, at which time the two beams can be slowly accelerated to full energy and made to interact at six separate experimental stations.

The proton collisions will provide a centre-of-mass energy of 800 GeV, equivalent to that available from protons of 340,000 GeV striking protons at rest. ISABELLE will therefore be able to extend by a factor of 10 the centre-of-mass energy range presently available on CERN's Intersecting Storage Ring; it will also have a far greater luminosity (the factor which determines the number of interactions).

ISABELLE has been on the drawing board since 1971, when preliminary designs were first made, and has enjoyed the consistent backing of the US high energy physics community. Official procrastination was broken last year by Representative Jerome Ambro, a Long Island congressman with one eye on science and the other on the jobs and money ISABELLE will bring.

After some political horse-trading that also involved the decision to site the Solar Energy Research Institute in Denver, Colorado, Mr Ambro persuaded Congress to add \$5 million to the Department of Energy's authorisation for 1978 to go ahead with ISABELLE—and to defeat attempts by the Office of Management and Budget to have the money deferred.

Further problems were raised when Dr Robert R. Wilson, director of Fermilab, tried to outbid Brookhaven for a major share of the 1979 budget in order to speed up the construction of Fermilab's Energy Doubler. However Wilson's hand was weakened when the department's High Energy Physics Advisory Panel reconfirmed top priority for ISABELLE.

The final hurdle came late in the spring, when the department announced that it had decided to maintain funding for high energy physics at a constant

level over the next few years—and that ISABELLE's construction period was to be extended to avoid initial "peaking" that would upset this scheme.

In political terms, the department hopes this will make the high energy physics programme more acceptable to a cost-conscious Congress. However, economically, the result has been to increase the projected overall cost of ISABELLE from \$240 million to \$275 million; and scientifically it means the extra two-year delay until the first results are produced.

"We would not have built this machine just to discover the W particle," Dr Jim Sandford, project manager for ISABELLE said last week. He points out, for example, that the increased luminosity over CERN's SPS ring, means that Brookhaven will be in a much better position for a detailed analysis of higher energy states, decay modes and so on.

"But I do not want to miss out, if at all possible, in finding it. With our greater luminosity, we had a good chance of finding the particle ahead of CERN on the five-year schedule. On a seven-year schedule, we have considerably less chance."

Brookhaven scientists still hope that, once construction of the accelerator's superconducting magnets and other components is well under way, the Department of Energy can be persuaded to alter its policy and make more money available earlier—saving on costs by reducing labour and avoiding some of the effects of inflation.

So far, the department has remained firm. It has proposed to make available \$45 million for ISABELLE's construction costs in the fiscal year 1980, making the total for the first two years still considerably less than the \$80 million which the laboratory had proposed for the first year of construction alone. □



Isabelle gets under way: Dale Myers, Assistant Secretary at the Department of Energy, in action at the ground-breaking ceremony

ESF warns of dangers of shortfall in posts for young scientists

"THERE is a potentially dangerous situation in Europe which is causing young scientists who might otherwise choose a career in research not to do so," Sir Brian Flowers, president of the European Science Foundation (ESF), said last week. He was speaking after the ESF assembly had approved the annual report for 1978.

A recent ESF study has shown that in most of its members' countries, "the majority of those now in employment will stay in office until the beginning of the 1990s when they will reach their normal retirement age. The demand for replacements during the next 15 years will be exceedingly low." The annual report says available posts are now so few that the chance of a junior research assistant getting a permanent university appointment has gone down from 70% in the 1960s to 15% this decade.

The problem of making posts available to young research workers is well-known. The ESF draft report adds one extra argument. In the past, it says, 65% of a university's budget was spent on personnel and the remaining 35% on maintenance, administration, upkeep of the library and research. Now the share taken by salaries has increased, leaving less for the other activities including research.

The ESF does not suggest what should be done. For the present it merely points out the dangers and urges countries to find their own solution.

The assembly approved a budget of 3.996m French francs for 1979. Among the activities it will finance are three new ones, two in the humanities—Byzantine and Chinese studies—and one covering the ESF's work in synchrotron radiation.

Ten member organisations are providing an additional 500,000 French francs for a feasibility study on building a European Synchrotron Radiation Facility. Such a machine would be distinguished from existing machines by its very high brightness, specially designed elements for each wavelength range, undulators which are very bright emitters and a beam time structure for time resolved studies. ESF hopes to complete its report within a year so a quick decision can be made whether to build the facility.

At the meeting of the Assembly, Sir Brian welcomed Dr John Goormaghtigh who takes over as Secretary-General from Dr F. Schneider in January 1980. Dr Goormaghtigh works in the social sciences.

Judy Redfearn

Wiesner attacks increasing government regulation of US university research

INCREASING attempts to regulate university research have led to floundering relationships between the federal government and the academic community that have reached a "point of crisis", according to Dr Jerome B. Wiesner, science adviser to President Kennedy and now president of the Massachusetts Institute of Technology.

Speaking at a conference of research administrators in Washington last week, Dr Wiesner said that this could seriously curtail the effectiveness of the nation's major research universities, and that the increasing intrusion of the federal government into university affairs threatened their capacity to produce innovative ideas.

He singled out for particular attack proposals published earlier this year by the Office of Management and Budget to introduce significant changes in the accounting procedures—currently listed in the so-called Circular 21—by which universities compute how much they should be reimbursed for the direct and indirect costs of carrying out federally-sponsored research projects.

OMB has claimed that the revisions are aimed at rationalising the procedures used by different government agencies, and reducing the risks of the possible misuse of funds. Dr Wiesner, however, said that the proposed changes, which have been widely criticised by the research community, would weaken universities as institutions and reduce their capacity to

conduct high quality research.

"There is no question, for example, that they limit in a destructive way reimbursement for indirect costs that are necessary and essential. There is no question that a number of the proposals are inequitable or administratively impracticable or both."

He called on President Carter to defer implementation of the revisions until a full study of the current problems faced by university research workers had been completed. "The proposed revisions to A-21 not only sacrifice flexibility but, far more significantly, they move in the direction of viewing universities in the same manner as commercial organisations and away from the concept of a partnership between the universities and the federal government."

Dr Wiesner also criticised the recent decision of Congress, in passing the National Science Foundation's budget for the fiscal year 1979, to place a ceiling of \$48,000—the maximum rate payable to Government scientists—on the salaries of university research scientists receiving university support.

"What this means is that Congress is limiting the reimbursability of salaries of the best faculty, the stars, the Nobel Prize winners, those people who make our institutions great. Universities will have to make up the difference, starting with an already substantial impact, and that's only the beginning," he said.

David Dickson

Few surprises in UNCSTD draft plan of action

A DRAFT plan of action has been published by the secretariat of the United Nations Conference on Science and Technology for Development (UNCSTD), scheduled to take place in Vienna next August. The plan will be discussed by the UN General Assembly in New York next week, and a revised version will then be considered by the conference's preparatory committee when it meets at the beginning of next year.

There are few surprises in the draft plan, which is essentially a synthesis of the various proposals made in the national and regional papers which have already been submitted to the secretariat (a number of countries—perhaps most noticeably the United States—have yet to complete their national papers).

The draft plan of action has reduced the proposals to 201 separate recommendations grouped into two main sections: science and technology

for development, and "institutional arrangements and new forms of international co-operation in the application of science and technology."

Popular themes include the need for codes of conduct covering both the transfer of technology and the behaviour of multinational companies, the need to improve scientific and technological information services and policy-making machineries, and concern about the effect of the "brain-drain" to developed countries. There are also suggestions for encouraging cooperation in research between developing countries, and ways of re-orienting educational systems towards national needs; and several countries ask for an emphasis on the development of appropriate technologies.

Other suggestions range from the proposal that developed countries set aside 0.5% of their gross national product for research related to Third World issues, to the suggestion that

regional "multinational" companies be set up to break the monopoly of foreign transnationals in selected areas of interest to a particular region—something that could be achieved, it is suggested, by polling the resources of a group of countries in the region and by arrangements for market-sharing, as is now done by the Andean pact countries of South America.

The draft plan says little about basic science. One of the few explicit references is in paragraph 63, which says: "One developing country and one market economy country recommend the fostering of basic research as an important basis for indigenous scientific and technological development." But the same paragraph continues: "Several countries recommend a more utilitarian rather

than an academic approach to the results of scientific research and underline the special importance of applied research and technological development for countries with limited resources."

Nor is much attention given to the obstacles to applying science and technology to development although they had been stressed previously by the conference secretariat. The introduction to the plan of action admits that "the approach by most governments in the preparation of their national papers has been to deal implicitly with obstacles throughout their papers."

As a result, the secretary-general of the conference, Dr Frank Joao de Costa, says that he has adopted the same approach in the outline of the

plan of action; and the introduction states that the number of obstacles treated explicitly "has necessarily been limited."

The two resulting paragraphs are vague and generalised. The first says merely that "systematic analysis and study" of such obstacles is a prerequisite for their elimination. And the second, in language somewhat typical of the whole document, states: "Many developing countries consider that the absence of an awareness of science and technology, often in the context of the social structure, cultural heritage, value system and religious traditions of a country may constitute an important obstacle to the application of science and technology."

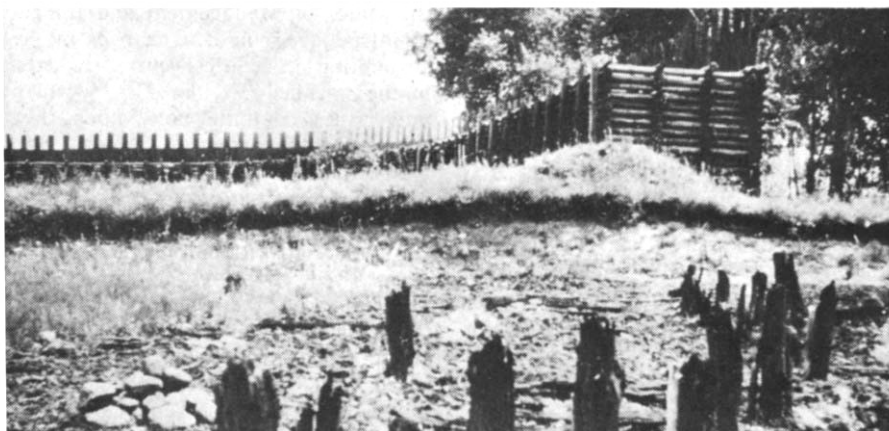
David Dickson

Problems of preserving a 2,500-year-old township

SINCE its discovery in 1933, Biskupin has posed a considerable problem to Polish archaeologists: how to excavate this prehistoric township whose timbers were preserved virtually intact in the peat without the immediate deterioration and decomposition of the finds once they are in contact with the air. A conference of wood conservation specialists, organised last September by the Polish State Archaeological Museum, which is responsible for conservation work at Biskupin, indicated, however, that during the last three years extremely satisfactory results have been attained at attempts at conservation *in situ*.

Biskupin, which dates from circa 550 B.C., was an island settlement of two-bedroomed wooden houses, arranged rectilinearly and enclosed by a palisade. Abandoned when the water-level of the lake rose, the houses collapsed and their timbers were preserved in the peat. The value of such a site for early iron-age archaeology is inestimable; however, until recently the problems of conserving the timber finds seemed insurmountable.

The new conservation programme at Biskupin uses a polyphenol resin to preserve the timber. The method is adapted from one introduced six years ago by Yurii Vikhrov, of Minsk, (Byelorussian SSR). In the Polish method the fluid is applied with a brush, and then cured by sunlight, temperature or catalyst hardening. Curing time depends



Reconstruction of the palisade with original wood in the foreground. Below: applying the solution.

on the conditions, but, using hot-air blowers, a typical figure would be 30 hours at 100 °C.

The resin penetrates the wood to a depth of 3 cm; this gives a mechanical strength that is quite sufficient for conservation; indeed, this method has already been used for the conservation of a whole mediaeval house at Breston-Bug. According to Teresa Stanczuk-Rozycka, head of the conservation laboratory of the State Archaeological Museum, the method, although expensive, nevertheless works out at only 20% of the cost of the method used in Sweden to conserve the timbers of the *Vasa*, and, unlike the Swedish method, is suitable for work *in situ*. Mrs Stanczuk-Rozycka further said that the September conference showed that the overall results of the method were extremely good, and that the programme of conservation would go forward.

At present, the conservation teams propose to work only on the remains of the gate and ramparts, excavated during the early digs, which are now under the greatest risk. With all future



excavations, however, running conservation of the wood in parallel to the actual digging work will be carried out as a matter of routine.

And, since the actual technique of applying the fluid and, if necessary, the hot air blowers, is extremely simple, a large team of skilled conservationists will not be necessary. "Anyone can do it", Mrs Stanczuk-Rozycka, told *Nature*, "for example, students!"

Vera Rich

Vietnam's scientists wait for books and equipment

AT THE HEIGHT of the Vietnam War, US Air Force chief General Curtis Lemay threatened to bomb North Vietnam "back into the Stone Age". Although American bombing never achieved this objective, it nonetheless destroyed a great many buildings, among them — factories, offices, hospitals and research laboratories. Today, in spite of extensive rebuilding office space is generally in very short supply, which means many scientists have to work in cramped conditions, particularly in Hanoi. In Ho Chi Minh City (formerly Saigon), things are not a great deal better, although it did emerge from the war less scarred.

Many of Vietnam's leading scientists have now moved to Ho Chi Minh City, under the auspices of the country's largest research establishment, the Vietnam Scientific Research Centre. Laboratories of experimental biology, petrochemistry, and physics and chemistry of natural products are already established in the city. The latest addition is to be a laboratory of biochemistry and food chemistry, early next year.

One incentive for moving south is the prospect of working in some of the better-equipped food and pharmaceutical industries. The great drawback is a shortage of scientific literature. According to one scientist, such material is in very short supply in Hanoi, but even this constitutes a "wealth much to envy" when compared with the south.

There have been reports of discrimination against scientists who worked for the south during the war. The latest information from Ho Chi Minh City however, suggests that northern scientists now in the south are making a major effort to bring their southern colleagues back into the fold. A major obstacle is the lack of available scientific equipment; the accommodation is there, but not the technical support, and it appears there is little the government can do to improve matters at present in the current economic conditions.

● The official British response to Vietnam is somewhat confusing. The Ministry of Overseas Development (ODM) and the Foreign and Com-

monwealth Office are known to be concerned with human rights in Vietnam, and new aid programmes are being refused until they are sure that no violations of rights are taking place. But in Vietnam itself, the British Ambassador has bought some £2,000 of much-needed scientific literature for biologists working at the Vietnam Scientific Research Centre in Hanoi. He is apparently allocating a further £2,000 for books next year.

● Back in Britain, neither the Royal Society nor the British Council appears to be doing much to promote contacts with Vietnamese scientists, despite discussions earlier this year about inviting one or more Vietnamese scientists to the UK. A Royal Society official said it was "into China at the moment". But a leading British scientist has urged the Society not to abandon its interest in the scientists of Vietnam who are in urgent need of assistance. The Royal Society, he said, could do much to help, simply by making and maintaining contact with the Vietnamese.

Alastair Hay

India gets ready to tune in to its satellite

THE building of seven ground receiving stations has begun as the first stage in preparing for the Indian National Satellite (INSAT) programme. The stations will be linked next March with a fractional transponder aboard an INTELSAT satellite above the Indian Ocean.

The INSAT project, costing six crore, is planned to provide remote areas with communications. By the time INSAT-I is launched in 1981, 28 more earth stations will be built all over the country.

INSAT-I is a multipurpose domestic communications satellite with a seven year lifetime to be built by the US Ford Aerospace Corporation. It will provide nation-wide direct television broadcasting to community receivers in rural areas, and continuous meteorological data in the visible and infrared.

Of the 35 ground stations to be built, six will be mobile, four by road and two by air. The one at New Delhi will be the biggest, acting as the control and monitoring station for the others. For meteorological studies about 110 unmanned data collection platforms will be installed at inaccessible spots. INSAT-I will give a cloud picture over the Indian Ocean, the Arabian Sea and Bay of Bengal every half hour.

As well as the Department of Space, other agencies like Posts and Tele-

graphs, Indian Meteorological Department and the Ministry of Information and Broadcasting will be responsible for the establishment and operation of the INSAT network. Most of the equipment needed to build the first seven earth stations will be imported, but Indian-made equipment will be used for subsequent stations. The total INSAT project will cost Rs 175 crore with a recurring expenditure of Rs 11 crores per annum. □

Third World seeks low frequency broadcasting

THE non-aligned countries should seek a suitable frequency for direct sound broadcasting via satellite from the World Administrative Radio Conference (WARC) when it meets next year. This was decided at a three day conference on "Satellite in Broadcasting" among the non-aligned countries recently held in New Delhi.

According to experts at the conference direct sound broadcasting via satellite would enable the member-countries to use relatively inexpensive and mobile receivers. The Indian Information and Broadcasting Minister Shri L. K. Advani said that high costs and the need for high-level expertise need not deter the member-countries from using satellites for communications. Costs could be reduced through

collective efforts, he said, and the technical expertise was already available in the developing world.

The purpose of the conference was to prepare a comprehensive report on the technical, economic, legal and political aspects of satellite broadcasting which will be presented at the forthcoming meeting in Tanzania of the Committee for Cooperation among the Broadcasting Organisations of Non-aligned Countries.

The experts felt that the selection of a particular mode of TV or radio transmission must depend on local conditions. They outlined a way of choosing the best mode taking into account the geography and available infrastructure of the individual country. They also decided to work out a way of exchanging TV and radio programmes among the member countries currently using the INTELSAT system.

Directly linking the domestic satellite of non-aligned countries is not yet possible because they transmit at high frequencies. The conference therefore agreed to urge the WARC to allot a frequency lower than 54 GHz — the lowest frequency band so far used. India is likely to ask for a long wave frequency because of the work she has done on long wave frequency propagation technology for low cost and long range broadcasts.

Dilip M Salwi

US carcinogen control threatened by costs

THE US Department of Labour's Occupational Safety and Health Administration (OSHA) seems to be losing the fierce battle that it has put up against being required to carry out detailed cost-benefit analyses of attempts to regulate occupational exposure to toxic substances.

OSHA director Dr Eula Bingham, previously professor of environmental health at the University of Cincinnati Medical School, remains adamant that such analysis is not required by the Occupational Safety and Health Act of 1969, beyond ensuring that no action threatens the overall survival of a particular industry.

OSHA is far from giving in to the attacks of those who claim that further regulation is merely aggravating the country's inflation problems. This week, for example, the Department of Labour was expected to announce a new standard for occupational exposure to lead of 50 micrograms per cubic metre of air—four times lower than existing permitted levels—despite its own studies showing that this will have a substantial economic impact on the lead-storage battery industry.

However, two recent events indicate the increasing pressure on OSHA to act not just on scientific evidence—or lack of evidence as the case may be—to protect workers' health but also to consider the economic implications.

The first is a statement from the Regulatory Analysis and Review Group, an offspring of the Council on Wage and Price Stability widely distrusted by environmental and labour groups. In its comment on OSHA's proposed scheme for classifying and regulating chemical carcinogens, the review group echoed opposition to the scheme by the US chemical industry, criticised its 'inflexibility' and claimed that the cost of implementing it would be "potentially quite substantial, well in excess of \$1 billion."

Similar pressures are also beginning to come through the courts. Last month, a federal appeals court in New Orleans set aside proposed new benzene exposure rules proposed by OSHA in June, on the grounds that the agency had failed to demonstrate a "reasonable relationship" between anticipated benefits and costs.

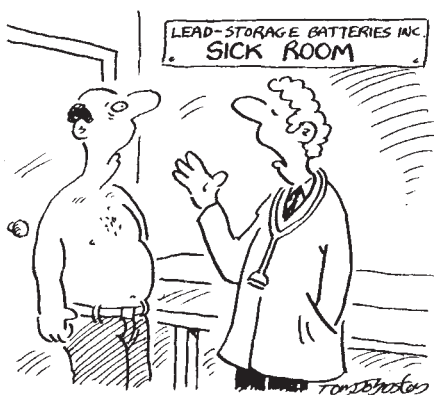
OSHA has already indicated that it will appeal against the court's ruling, a significant departure from earlier legal decisions which had accepted that such cost-benefit analysis was not necessary. But it accepts that if the ruling stands, then it would be required to shift its strategy; "if one has enough interpretations that this is demanded, then

one would do it", Dr Bingham said.

The agency's reluctance to engage in such analysis seems based on three fears about how it might undermine OSHA's mandated efforts to protect the health of US workers. The first is that a requirement to carry out detailed cost-benefit studies would overburden an understaffed agency.

The second fear is that, as far as information on costs is concerned, OSHA would have to rely heavily on data provided by the industry itself, which it feels might be overstated.

Thirdly there is a feeling that occupational health is an area in which scientific uncertainties are so high that any attempt to place definitive values on degrees of risk, etc., would merely disguise how such judgments reflect broader social values, and that these



"You're suffering from a bad case of Federal Appeals Court decisions, aggravated by inflation problems!"

could be better dealt with if openly acknowledged.

"There is in scientific terms no evidence of a 'threshold'. But there is evidence that as you reduce the exposures, you are reducing the risk. If there are a few fibres or molecules left, theoretically someone somewhere might get cancer from it. At that point a social decision has to be made," according to Dr Irving J. Selikoff director of environmental sciences, Mount Sinai Hospital, New York.

The problems caused by the scientific uncertainties were illustrated by the public controversy last month over a statement released by the National Cancer Institute, the National Institute of Environmental Health Sciences, and the National Institute for Occupational Safety and Health, claiming that up to 20% of all cancer deaths might be attributable to occupational exposure.

Erroneously described by the US media as the results of a "new federal study", and frequently hardened to the claim that 20% of cancers were

attributable to such exposure, the report was in fact a position paper put together rather hurriedly to meet OSHA's deadline for comments on its carcinogen proposals—and was given premature publicity when Labour Secretary Ray Marshall quoted extracts at a trade union conference.

The main message of the statement is that earlier estimates suggesting one to five per cent of cancers can be attributed to occupational exposure appear to be "unrealistically low".

However, the American Industrial Health Council, an industry-sponsored group which has been leading the attack on OSHA's carcinogen proposals, criticised the 20% figure as based on "unwarranted speculation".

Emphasising the council's support for the one to five per cent estimate, Dr Fred Hoerger, chairman of the AIHC's scientific committee, said that "the predicted massive increase in cancer due to occupational exposure in the study is not supported by current knowledge of cancer rates, worker exposures, or numbers of workers exposed".

The debate has demonstrated the gulf that exists between the scientifically conceivable (represented by the NCI position), and the scientifically demonstrable (on which AIHC argues regulation should be based).

It is a gulf which OSHA feels would be prematurely foreclosed by translating the debate into one of costs against benefits, given the problems of adequately defining either. Industry has been impressing on the president's economic advisers that, with rapidly rising production costs, this is the only reasonable basis for legislation.

OSHA has so far taken a firm stand against being required to adopt industry's perspective. And it has suffered virulent attacks, such as the American Conservative Union's "Stop OSHA" campaign, for doing so. But it is not yet prepared to throw in the towel.

David Dickson

Correction

On page 108 of last week's issue a small error may have caused some confusion in interpreting the UK's proposed new guidelines for genetic manipulation. In the third column, item 4, the experiments referred to are those listed under the third item in the box on pages 106 and 107, that is those in Category 1* "for which at present there is no substantial evidence of risk. The chief candidates of this kind are the experiments in which *E. coli* genes are cloned in *E. coli* organisms".

news in brief

● **US Academy confirms saccharin as potential carcinogen:** THE US National Academy of Sciences has confirmed that the artificial sweetener saccharin can cause cancer in laboratory animals, and that it is probably a 'low potency' carcinogen in humans.

In a report prepared at the request of Congress, which last year imposed an 18-month moratorium on a proposed ban of saccharin by the Food and Drug Administration, an academy review panel says that "even low risks applied to a large number of exposed persons may lead to public health concerns".

The panel says that saccharin may also enhance the potency of other cancer-causing agents, and suggests that it may in fact be more dangerous as a 'promoter' of other carcinogens than as a carcinogen itself.

The panel also expresses concern at the increasing quantities of saccharin being consumed by young children, mostly in diet soft drinks. The length of time which cancer takes to develop could mean that such children have a magnified chance of developing cancer later in life, the panel says in its report.

● **Reprocessing plant still closed:** Employees at the Windscale reprocessing plant are still waiting for British Nuclear Fuels Ltd and the Nuclear Installations Inspectorate to decide when to re-start the reprocessing of nuclear waste. The plant was closed two weeks ago after a potentially explosive build-up of hydrogen in one of the silos where magnox cladding material is stored. In a reply to questions from the conservation group Friends of the Earth, the Department of Energy said workers were not in danger from the release of radioactivity from the silo, but reprocessing would not restart until an investigation revealed the cause of the build-up of hydrogen and if any measures needed to be taken to prevent a recurrence. A number of questions remain to be answered. For example, why, after 15 years of storing magnox in this manner, has the build-up of hydrogen not occurred before? A spokesman for British Nuclear Fuels told *Nature* that they wanted to be sure they understood what had happened before restarting reprocessing.

● **US judge claims low levels of PBBs "not toxic".** A county circuit court judge in Michigan has ruled that the low levels of polybrominated biphenyls (PBBs) to which cattle in the state were exposed after fire retardant had been mistakenly mixed with animal feed, were "not toxic". As a result, he dismissed a claim for damages issued by a Michigan farmer who slaughtered his herd on realising that they had eaten contaminated feed provided unknowingly by the Farm Bureau Services, a farmers' cooperative, in 1973. In a 177-page opinion handed down after the longest trial in state history, Judge William Peterson said that the animals had been slaughtered needlessly because of public fear aroused by "incompetent and dishonest" scientists, veterinarians and lawyers who, he claimed, were trying to capitalise on the disaster.

Fears about the potential carcinogenicity of PBB have been raised because all other halogenated aromatic hydrocarbons tested to date have turned out to be carcinogenic. Research on PBB is now being carried out at the National Cancer Institute in Bethesda, Maryland. However, referring to the arguments of those who had warned of the potential long-term hazards of low-level exposure to PBBs, Judge Peterson said: "Professionals have forsaken objectivity and their usual standards of inquiry to accept unquestioningly,

as a basis for their expressed opinions, reported facts that were not factual."

● **\$1.5 million to erect statue of Einstein:** The US National Academy of Sciences announced this week that it is to erect a statue of Albert Einstein, three times life-size in the academy's grounds on Washington's Constitution Avenue. The statue, which will be unveiled next spring in a ceremony honouring the centennial of Einstein's birth, will be carried out by Robert Burks, who sculpted a portrait head of the physicist in 1953. The cost of the statue, the base and the ground preparation will be more than \$1.5 million. A fund-raising campaign is being organised by the academy among the US technical community.

Announcing the plans for the statue this week, Dr Philip Handler, president of the NAS, said: "It is our hope that this statue will serve several public purposes—as a tribute to the enormous analytical and creative powers of this unique scientist to whom we owe so much, as an evocation of his profound spirituality and his concern for mankind, as a reminder that Einstein found in this country refuge from tyranny, and finally as an appreciation of the accepted place of science in our civilisation."

Meanwhile, in Europe, two interdisciplinary scientific meetings in Munich and Ulm marked the beginning of an international series of meetings to be held throughout 1979 to commemorate Einstein's birth on March 18, 1879. At the meeting in Ulm, Paul Dirac, who worked with Einstein at Cambridge and Princetown and who was awarded the Nobel Prize for physics in 1933, paid a tribute to "the excellence of Einstein's theory of gravitation".

● **Indian shuffle:** The new Secretary in the Indian Department of Science and Technology is Professor M. G. K. Menon. He will also serve as director-general of the Council of Scientific and Industrial Research. These posts became vacant with the departure of Dr A. Ramachandran to head the UN Centre on Habitat in Nairobi. Professor Menon is at present chairman of the Electronics Commission and secretary to the Department of Electronics and was for several years scientific adviser to the Minister of Defence. Well-known in the international science policy world, he has served as a member of the UN Secretary-General's Advisory Committee on the Application of Science and Technology to Development. His research in the fields of cosmic rays and elementary particles was largely done at the Tata Institute of Fundamental Research, of which he was director from 1966 to 1975.

● **Britain joins in China exchange:** China is continuing to expand its scientific cooperation with western countries. Up to 100 Chinese research workers are to come to UK scientific laboratories for periods of about four years, under the terms of an agreement between the Royal Society and Mr Hu Ke-shih, vice-president of the Chinese Academy of Sciences, which was signed on 10 November. Mr Hu was leader of a delegation of scientists and Academy administrators who have just concluded a three-week visit to the United Kingdom as guests of the Royal Society. The group visited many university departments and research establishments such as the Rutherford Laboratory at Chilton and the Imperial Cancer Research Fund Laboratories in London. The agreement comes soon after a similar deal to exchange post-graduate students between China and the United States.

Should scientists attend the ICUS?

● When the third International Conference on the Unity of Sciences (ICUS) was held in London in November 1974, scientists were made aware of the link between the International Cultural Foundation, the conference organisers, and Mr Sun Myung Moon, the head of the much criticised Unification Church (Nature 251, 661; 1974). Since then, scientists have been divided over whether to attend subsequent sessions of ICUS. Here, Professor N. Kurti of the Department of Engineering Science, Oxford University explains why he feels justified in attending them.

THE seventh International Conference on the Unity of Sciences (ICUS VII) will take place in Boston, Mass., 24–26 November, 1978. No other series of scientific conferences has engendered so much passion, largely because these conferences have been financed and run by the International Cultural Foundation, one of the 50-odd organisations founded and presided over by Mr Sun Myung Moon, the wealthy, and passionately anti-communist South-Korean industrialist and businessman.

Wealth, strong political bias, success in business are not normally considered unacceptable characteristics in sponsors of conferences, but Mr Moon has one further attribute which is at the bottom of the animosity which the activities of the Moon-organisation create: he is the founder and head of the Unification Church which has hundreds of thousands of followers in South Korea and Japan—though its active membership in Western Europe and North America is probably well below 15,000. He is regarded by his followers as the new Messiah called by God to finish the work begun by Jesus Christ—and to my knowledge has never made an explicit disclaimer.

The Unification Church has been strongly attacked for its forceful proselytising activities and accused of brainwashing young people and alienating them from their families. It has many aspects which I find distasteful. The following excerpts from an interview Mr Moon gave to American Newsweek International are revealing: “God has hand-picked me” (The basis of an intense personality-cult). “The line-up in Panmunjon is like the line-up between the heavenly world and the satanic world” (President Park’s South Korea seems to me a strange sort of heaven). “If one of my disciples denounces his parents he is not really my disciple” (How many of the Moon-disciples who did break with their families were expelled from the Church?).

It is not surprising that the following two questions should have been asked often: can a conference sponsored by a militant and politically motivated organisation which many regard as a danger, not only to the spiritual well-being of young people, but to the structure of society as a whole, do its work in an unbiased, objective fashion without being influenced by the views and beliefs of its sponsors? Are the scientists, among them many world-famous figures, allowing themselves to be used as propaganda material for the Unification Church? Having attended five of the six conferences held so far I shall try to answer these questions.

The first conference, organised by Mr Edward Haskell, author of the book *FULL CIRCLE, The Moral Force of Unified Science*, and held in New York in November 1972 was a dismal affair. This is not surprising, since the above book, which formed the basis of the meeting, contained such choice proposals as periodic tables for the “Plant Kingdom”, the “Animal Kingdom”, “Human Society”, etc. After all if science is “unified” then what goes for chemistry goes for other disciplines!

Such was my disillusionment with this first ICUS that in the summer of 1973 I declined an invitation to attend the second conference in Japan. I did the same when I was asked a year later to attend ICUS III to be held at the Royal Lancaster Hotel in London. However when I noticed that several of my colleagues for whom I had great respect were associated with the conference and had helped to shape its programme, I accepted, and did not regret it. ICUS III, together with ICUS IV held in New York in 1975, were, in my view, the best of this series.

One might well ask whether anything worthwhile can come out of conferences devoted to the unity of sciences—science being used in the wider sense of the word, equivalent to the German *Wissenschaft* or the Russian *Nauk*. Those who search for or want to believe in a ‘unity of sciences’ are often tempted to transfer concepts appropriate to one discipline to a totally different one and proclaim the result as a proof of ‘unity’. Mr Haskell’s periodic tables for botany, zoology, sociology are examples, so are Mr Moon’s speculations about the origins of energy given in his address to ICUS V: “Without there first being a subject and an object pair, it is impossible to produce energy. That is to say, the relationship of a subject and object is indispensable as in the case of an atom where the proton (which is

the subject) and the electron (which is the object) must both be present before beginning to interact”. Hence “Energy is the phenomenon resulting from the process of the two (subject and object pair) becoming one”. The rest follows: happiness is the result of “mind and body being united in one”. Illness is the result “of the subject and object elements of the body failing to attain unity and harmony”—the medicine prescribed by the doctor “restores the harmonious unity”.

If ‘unity of sciences’ is an ill-chosen aim of this series of conferences, the choice of the titles of the individual conferences was equally poor. What can one make of ‘science and absolute values’ (ICUS III), ‘the centrality of science and absolute values’ (ICUS IV), ‘the search for absolute values: harmony among the sciences’ (ICUS V)? But fortunately conferences are not determined by titles and slogans but by the quality of the participants and their interests, and even meaningless clichés can lead to useful conclusions and the crystallisation of ideas.

The following examples may illustrate this point. At ICUS III in London one of the four committees spent two days on lectures and discussions under the general heading ‘the idea of the university in a troubled world’. One of the papers had the daunting title ‘international education: a search for cultural and conceptual isomorphisms’, but fortunately it had as an explanatory motto a quotation from Mark Twain:

“I went next to the Secretary of War . . . I said there was nothing so convincing to an Indian as a general massacre. If he could not approve of the massacre, I said the next surest thing for an Indian was soap and education. Soap and education are not as sudden as a massacre, but they are more deadly in the long run; because a half-massacred Indian may recover, but if you educate him and wash him, it is bound to finish him some time or other. It undermines his constitution; it strikes at the foundation of his being.”

There was a lively discussion by philosophers, historians, economists, physicists, etc., on the question of whether or to what extent one civilisation should impose its system of education on another. As one participant said: “Is the educational system developed in Europe during many centuries fruitful for the Bantu child in a surroundings of tribe-togetherness?” Another paper under the same general heading was entitled ‘cognitive limitations and public decision making’ and led to an examination of the relative roles of intuition and statistical analysis in decision making.

As in ICUS III so in ICUS IV (New

York) the interaction between developed and developing countries came under scrutiny. Thus the Committee which discussed 'the university, research institutions and society' returned again and again to that particular question, as the following examples show:

- Should universities in developing countries follow the principle enunciated in 1896, "only the best is good enough for Africa", or, should they eschew "excellence" in favour of "relevance" and be more attuned to local needs and aspirations?

- There seemed to be a need to scale down well-established technologies to suit developing countries, so that they are not forced to buy needlessly large plant, because nothing smaller was available.

- How can developing countries be persuaded to accept "intermediate technology" and not feel that they are being "fobbed off with the second best"?

I believe that even the severest critics of these conferences, agreed that the lectures and discussions were free of constraints, and that the proceedings of the conferences were not censored at all. The best proof can be found in Professor A. J. P. Martin's controversial lecture given at the closing session of ICUS III. One of his points was that there are too many people around, and that the population of the world, should be reduced by a factor 10. He proposed, for instance that the prison population should be reduced by submitting the inmates to carefully dosed X ray treatment which drastically shortens the expectation of life. These and similar suggestions, for example about selective breeding of humans, cloning etc., were delivered in deadly earnest, and I just could not judge how much of it was meant seriously and how much was "*pour épater les bourgeois*", or rather "*les moonies*". But what I find important is that Professor Martin's lecture, all of which was anathema to Unification Church tenets was printed in full in the proceedings.

It cannot be asserted that what was said at the unity of sciences conferences could generally be used in support of the Unification Church, but it is arguable that who said it could be of propaganda value. Indeed there have always been many Nobel-Laureates among the participants—at ICUS III in London 25 out of 140—and most ICUS Chairmen were Nobel Laureates: Lord Adrian (ICUS III), Professor R. S. Mulliken (ICUS IV), Sir John Eccles (ICUS V and VI), Professor E. P. Wigner (ICUS VII).

It is often said that the presence of so many Nobel Laureates and other well known scientists at the conference

could be used to enhance the "respectability" of the Unification Church, and to neutralise the attacks made on it. Such fears are supported by the continuous tape-recording, filming and photographing at these conferences. But the remedy is often in the hands of the participants. When after ICUS VI the organisers were compiling a little glossy brochure about these conferences to lessen the effect of hostile press comments, participants who were to be quoted were asked for their agreement. I thanked the organisers for their courtesy, said that I regarded these conferences as *bona fide* scientific affairs which should be judged by the contents of the published conference reports and not by selected remarks of participants and others. My request not to be quoted was complied with.

To what extent are these conferences



1974—ICUS meets in London. At the microphone: Professor Archer Martin.

truly "international"? In 1975 I pointed out to the organisers that a conference to which people from the Communist countries are apparently not invited and which, while having quite a few participants from Israel, has none from Arab countries, could not be called "international". I made certain concrete suggestions and since then there is representation, albeit small, of both Arab and Communist countries.

It is sometimes said that these conferences are used to make propaganda for the Unification Church and that the programme is arranged and the speakers chosen to suit the Church's book. My personal experience does not bear this out. As a member of the International Advisory Committee I frequently suggested speakers and participants whose religious and political ideas were far removed from those of the Unification Church, and yet my suggestions were invariably accepted. Of course many of the participants, among them scientists of considerable reputation, admire Mr Moon and the Church, but this does not interfere with their scientific judgment and in fact the Church, and for that matter Mr Moon, are hardly mentioned if at all.

I shall now answer the two questions I posed at the beginning of the article. The ICUS are *bona fide* scientific conferences free of political and religious bias. Not everyone may agree with the choice of topics, but this is not surprising for conferences which draw their participants from disciplines spanning most intellectual activities.

The second question, namely to what extent one's participation at ICUS furthers the aims of the Unification Church falls into three parts.

Has it any propaganda value? The films and recordings made at ICUS are, I believe, shown at Unification Church centres and functions, and it could be that the Church's aspirants and disciples are gullible enough to believe that the conference participants are *ipso facto* supporters of their Church and that the Nobel Laureates photographed with Mr Moon are his followers. Against this must be said that the conferences give ample opportunity for talking to the press, radio and to members of the Church, and many participants make it clear on such occasions that they have nothing to do with the Unification Church—perhaps even making critical remarks about it. For them ICUS is simply a multi-disciplinary scientific gathering.

Does acceptance of the fare and full board put one under an obligation to the International Cultural Foundation and to the Unification Church and colour one's attitude to them? If this were so, then scientists who regularly receive funds from many sources would be among the most diversely bribed members of the community owing allegiance to a multitude of organisations. Is the integrity of scientists really rated so low?

Can attendance at a conference sponsored by or connected with a religious or political organisation of whose activities one disapproves be morally justified? This is similar to the perennial question: should one attend conferences in countries with whose political system one disagrees, or which persecutes some of its citizens? There are those who say "No". For instance, there were some French people who even several years after the German occupation could not bring themselves to set foot on German soil.

But others say that adherence to such rigid principles, would make international scientific exchanges and collaboration impossible. One must follow one's conscience, and I personally have no moral qualms about attending an interesting and useful conference provided I am satisfied that in doing so I am not furthering aims or activities which are regarded as undesirable or even harmful. □

correspondence

The fate of the Taj Mahal

SIR,—M. K. Agarwal has made a plea (2 November, page 10) that India should go ahead with the construction of the proposed oil refinery near the Taj Mahal and asks whether a poor nation can afford the luxury of keeping its monuments in their pristine beauty while it cannot provide a minimum for its people. While the intentions are laudable, I would like to contest the rationale.

If the shifting of the proposed site of the refinery by a few hundred miles is really going to affect the economic development of India to a significant degree, Mr Agarwal might indeed have a case. If there are scores of other marble monuments across the country which are all threatened by projects vital to reduce human misery, he may again be justified in criticising the efforts to save the Taj Mahal from corrosion, as one cannot go around cancelling all development schemes.

While changing the site of the refinery will only cause a bit of a headache for the planners and the bureaucrats, I cannot see much harm even in completely dropping the proposal. Looking through India's record since independence, no sincere observer can escape the fact that despite her impressive technological progress, the percentage of the population under the starvation line has remained around 60%. In absolute numbers, poverty has only increased.

The massive nuclear reactors, steel factories, refineries and the rest have benefited mostly the affluent and the urban middle-class. The majority of poor rural India, which should be the primary concern of any development project, has hardly gained from the industrial growth. In fact a case can be made that this western style technological progress has done more harm directly by increasing the stranglehold of the big industrialists, and indirectly by diverting valuable money from investment in the rural sector and in appropriate, labour-intensive, small scale technology. One could also argue that without fundamental social changes and agrarian reforms, any industrial progress in India can only be harmful.

Rather than being an economic liability, the Taj Mahal has always brought enormous foreign exchange to India. No tourist leaves the country without visiting Agra. Even commercially, it would be wiser to back the Taj Mahal rather than the refinery. Under these circumstances, the effort to save it from corrosion is hardly a luxury for a poor country.

If the construction of the oil installation goes ahead and the pollution does damage the monument irreparably, posterity will never condone this generation for sacrificing one of mankind's great architectural wonders for a very dubious (perhaps despicable) economic reason.

T. R. VIDYASAGAR

UMIST,
Manchester, UK

Discussing values and judgments

SIR,—I was most interested in your editorial comments of 20 July and feel constrained to address some of the inconsistencies they contain. In the first place, the separation of scientists from "the others" in terms of their ability to discuss values and human judgments and to deal with "convergent" rather than "divergent" problems seems to be both misleading and patently false.

During my years at university, I was constantly made aware of the barriers erected between the arts and sciences and was frequently told that "you are unable to understand these issues because your scientific training hasn't given you the necessary foundations for understanding general cultural and value systems." Rubbish! What I have observed during my years in university and subsequently is that non-scientifically trained graduates tend, if anything, to be a bit more dogmatic in their beliefs than scientists and are often unable to carry on philosophical discussions in a logical and consistent manner.

My education prepared me to make value judgments and the scientific method has fitted me admirably to address open-ended problems in a reasonable way.

All of your analysis seems to address reaction to recent evaluations of the Windscale inquiry and, specifically, the remarks of Professor David Pearce. You make further reference to the fact that the nuclear industry appears unwilling to discuss values—including the fear of nuclear weapons proliferation. Having chosen to "leave" such speculation (with which term you correctly identified your ramblings), I should like to judge on its own merit your recommendation that school children be politically educated by requiring study projects linked with technological issues, such as the need for fast breeder reactors. In this way, you suggest, the humanist and the scientist (again, this false separation of the two aspects of each of us) can learn from one another. Assuming that you are referring to pre-university education, I very much doubt that your desired goals will be reached, since it is unlikely that such students are well enough prepared for the kind of in-depth study required to achieve these ends.

I should like to make further comment concerning the remarks of David Pearce in the following discussion of the values which are at the root of the nuclear debate. His statement proposing that opposition to nuclear power derives from differences in value systems strikes at the heart of this controversy; however, as I read them, his comments suggest that the "image of infinite credibility" is a cloak worn only by the nuclear industry and that they, so deceiving themselves, stand in the way of clear discussion of the values which lie beneath the issues discussed at Windscale. In a way this is rather humorous for, from my position on the other side of the controversy from

that taken by David Pearce, it appears that this cloak is being worn by opponents of nuclear power development. I was glad to see him admit that there were many "men of reason" characterising the pro-nuclear stance but the impression given in his article was that this was the exception rather than the rule. With this same proviso, I could change the term 'industry' into the term 'environmentalist' and make the entire article read very much to my liking—perhaps proving, as Pearce suggests, that the debate has fallen to a very low level indeed.

A clue to the problem (which is raised by discussions such as the one presented by Pearce) is revealed when one considers the 'problem' of defining public accountability for decisions made in a social context. Institutions must be modified to accommodate other points of view, states Pearce, with the thinly-veiled threat that failure to do so will result in conflict (presumably including physical violence). I suggest that the real problem in the nuclear debate is the fact that institutions have been modified to the point where everyone, no matter where his expertise lies, presumes himself fully-qualified to speak on every issue and, for this reason, totally irrational points of view are given prominence simply because of their sensational value in the public eye.

Heaven help our society if orderly inquiry into questions such as that recently carried out at Windscale is replaced by mob rule because people such as David Pearce are unprepared to accept defeat when their values are weighed in duly-constituted public inquiry and rejected. Justice Parkers' report seemed a well-reasoned and impartial survey of the many questions brought up at the inquiry and the fact that he dismissed out of hand a number of alternative value systems proposed by some of the intervenors is not a result of his failure to understand but simply a judgment based on his understanding of the will of the people of Great Britain as expressed through past legal decisions and acts passed by their elected representatives in Parliament.

R. C. FORRESTER III
Kingsport, TN, USA

Food production has kept up

SIR,—I was disappointed to see in your pages (26 October, page 698) the myth that there has lately been an "inexorable increase in the world's population without a commensurate increase in food supplies". FAO data show that during the past two decades of rapid population growth, while population has grown at 2% per annum food production has grown by an average 2.8% per annum. These results have been discussed in detail in *World Futures* (C. Freeman and M. Jahoda (eds), Martin Robertson, London; 1978).

JOHN GRIBBIN
University of Sussex,
Brighton, UK

news and views

Powder metallurgy: new techniques, new applications

from Robert W. Cahn

CERAMIC objects—crocery, fine porcelain, furnace tubes, insulators—are made by starting from a powder, binding it (with water or adhesive), sometimes pressing the mass and then firing. The bulk of the powder never melts (though part of it may); by a series of physical processes including bulk and interface diffusion, the particles weld together, or sinter, and the pores between them close up. There is really no alternative, because most ceramics are too refractory to melt and, being brittle, the powder particles cannot be squeezed together.

The idea of adapting sintering methods to metals seemed eccentric when it was first tried in Austria early this century, and the German term 'Metallkeramik' showed the origin of powder metallurgy as a variant of ceramic procedures. From the beginning, the attraction of powder metallurgy has been economy: a fairly complex shape can be made quickly and cheaply, without the problem of solute segregation inseparable from all forms of casting. In some special instances, powder metallurgy can create a product that cannot be matched by traditional means: the manufacture of long-lasting incandescent lamp filaments is the prime instance. (The progressive improvement of electric lamps, largely a matter of metallurgical ingenuity, is a fascinating chapter in the history of metallurgy). Apart from such special cases, powder metallurgy has generally been restricted to unsophisticated products, subject to modest demands.

All this is in the throes of change, and powder-metallurgical methods are beginning to be applied to demanding functions such as gas turbine disks and highly stressed airframe components. The reason is to be found largely in two very recent technical developments—rapid solidification processing and hot isostatic pressing.

Rapid solidification processing (RSP) is an American industrial development stemming directly from the techniques

of splat-quenching of alloy ribbons from the melt (see *Nature* **252**, 100; 1974). An alloy melt is atomised and sprayed through a helium atmosphere and the fine droplets, usually in the range 25–100 μm diameter, are frozen very rapidly; the exact cooling rate is subject to many experimental variables, but an average rate of $10^6\text{ }^\circ\text{C s}^{-1}$ is quite accessible. The particles are then pressed and sintered and may subsequently be extruded or wrought in other ways. This technique of making final products has the advantages that macrosegregation is entirely eliminated, large amounts of solute can be held in metastable equilibrium which makes possible new levels of precipitation-hardening, and the consolidated product can have very fine grain sizes which in turn leads to great ductility at ambient temperature—even to superplastic behaviour.

The method is being applied to superalloys for jet engine parts, titanium alloys for airframe components and to aluminium alloys. It has been difficult to discover much about these developments, many of them shrouded in industrial secrecy—but publication has now begun. Much information about both powder quenching and consolidation is to be found in the proceedings of a conference on Rapid Solidification Processing.* The other up-to-date source is a review article by N. J. Grant, presented at the Third International Conference on Rapidly Quenched Metals, Sussex University, in July 1978; this will be published in the *Proceedings* by the Metals Society, London, in December 1978.

Hot isostatic pressing (HIP) is a new technique, pioneered in Sweden, with even wider potential. A chamber (which may be a fair part of a cubic metre) is subjected to high temperature—it can be well above $1,000\text{ }^\circ\text{C}$ —and high uniform (isostatic) pressure—typically 1 kbar—transmitted by argon. The maintenance of such temperatures and pressures in such large volumes represents a technological achievement of a high order, with correspondingly high demands on safety precautions: in the

early days of HIP an operator was suffocated in a sea of escaped argon.

Specimens for HIP consist of loose powder contained in a sealed evacuated can of metal foil or in a shaped die of glass or steel, and the process converts them in one operation into pore-free solids. Ordinary sintering of metals, without pressure, will not reduce porosity below 3–4%, and removal of that small porosity leads to a large improvement in strength, ductility, toughness and fatigue resistance at room temperature. A related function of HIP is its use for 'rejuvenation' of turbine blades. Creep of a cast blade in service generates porosity at grain boundaries, which limits creep life; HIP can entirely remove this porosity and thus effectively confer on the blade a new lease of life. This method is just beginning to be used in practice. A further development, in America, is the 'near-net shape' process. A shaped thick die of glass or steel is used to contain the powder: at high temperature and pressure, the die behaves as a quasi-Newtonian fluid and transmits hydrostatic pressure. Complex airframe components can be made from powder—which may have been made by RSP—directly as 100% dense shapes which require only a minimum of machining.

The combination of RSP and HIPping (the neologistic verb is firmly entrenched in America) offers, for the first time in many years, some prospect of enhancing the service temperature of turbine blades. This is because the problem of macrosegregation in castings has strictly limited the permissible alloy content of superalloys; RSP has removed this limitation and a new range of alloy compositions is now accessible. The main problem is that the fine grain size produced by RSP, desirable for applications at ambient temperature, is quite unacceptable for turbine blades—which must have very coarse grains. There are indications

*Conference on Rapid Solidification Processing, Principles and Techniques, November 1977, Reston, Virginia, proceedings now published by Claitor's Publishing Division, Baton Rouge, Louisiana.

that it may be possible to achieve such grains by recrystallisation after RSP followed by HIP, without loss of the precipitation-hardened microstructure that confers hot strength. In this connection, current theoretical work aimed at identifying the plastic processes that enable pore closure during HIP-ping may prove important, because the feasibility of recrystallisation is closely linked to the amount and nature of plastic deformation previously undergone by the metal. Activity in this field is very intense.

Yet another potentially very valuable use for HIP has just been proposed by U. Engel and H. Hübner of Erlangen, Germany (*J. Mater. Sci.* **13**, 2003; 1978). They HIP-ped, at 1 kbar and 1,260–1,360 °C, samples of cemented carbide (tungsten carbide particles cemented by films of metallic cobalt, made by conventional sintering combined with partial melting). These very brittle materials are used as tips for lathe tools subject to exceptionally severe demands. It was found that, after

HIP-ping, all residual pores were completely eliminated, being filled by cobalt, which is itself not brittle. It appears that under HIP conditions the cobalt binder can flow freely between the carbide grains. Sophisticated methods of fracture testing were employed to examine the effect of HIP-ping on the resistance to brittle fracture, and it was found by statistical (Weibull) analysis that the mean fracture strength could be doubled, with a concomitant reduction in scatter of strength values. A critical comparison of observed flaw sizes with those computed from measured fracture strengths proved that the specimens were behaving as ideal brittle, flaw-limited solids both before and after HIP-ping. This very careful study indicates that HIP of cemented carbides should greatly increase the performance and life of lathe tools; moreover this should make economic sense as a production treatment, since cemented carbides are materials of high specific value. □

Megagauss magnetic fields in laser-produced plasmas

from M. H. Key

A recent paper by Raven, Willi and Rumsby (*Phys. Rev. Lett.* **14**, 554; 1978) describes the observation of an intense (1.8 MG) toroidal magnetic field surrounding the 30 µm focal spot of a pulsed (100 ps) laser focused on to a solid surface in vacuum. While it is intuitively obvious that $>10^{16}$ watt cm⁻² laser intensity will create a hot plasma in the focal region, it is much less obvious that a toroidal magnetic field will be generated with a growth rate of 10^{16} G s⁻¹. Indeed it was not until 1971 that Stamper *et al.* (*Phys. Rev. Lett.* **26**, 1012; 1971) of the US Naval Research Laboratory (NRL) detected kilogauss magnetic fields with magnetic pick up coils 1 ~ cm from a laser-produced plasma, and gave a basic explanation of the field generation mechanism. Before this it had often been assumed that a desirable feature of laser-produced plasmas was the absence of magnetic field effects!

The pick up coils used by Stamper *et al.* measured the field only in the tenuous plasma resulting from free expansion into vacuum about $\sim 10^{-7}$ s after its creation and it was not possible to deduce the much larger field strengths occurring in the microscopic laser-produced plasma itself. The question was nevertheless of great interest because it seemed that the field strengths might be great enough to

considerably modify the plasma behaviour with important implications for the developing ideas of laser fusion.

It was the NRL workers, Stamper and Ripin (*Phys. Rev. Lett.* **34**, 138; 1975), who tackled this problem by looking for, and observing in 1975, the rotation in the plasma of the plane of polarisation of a probe pulse of laser light by the magnetic field induced Faraday effect. Their results, indicating megagauss fields, were of a preliminary nature and subsequent efforts to reproduce them both at NRL and elsewhere were negative.

There developed a situation in which theoretical models of increasing sophistication such as that of Colombant and Windsor (*Phys. Rev. Lett.* **38**, 697; 1977) predicted the megagauss fields but only the doubtful NRL experiment had shown any direct evidence for the fields, and in particular very careful 4th harmonic optical probing experiments by Attwood at the US Lawrence Livermore Laboratory had shown no fields above 100 kG to be present when ~ 50 µm spherical glass shell targets were irradiated.

The paradox was resolved in 1978 when the NRL group devised a novel way to avoid a persistent problem of the optical probing experiments. 2nd or 4th harmonic generation using a fraction of the main laser pulse had hitherto been used as a convenient source of a synchronised optical probe pulse but the measurements were complicated by the emission

of those same harmonics from the plasma itself. The solution found was to generate the 0.53 micron second harmonic but to further shift its wavelength by stimulated Raman scattering in ethanol to 0.6329 micron. This eliminated interference from plasma generated harmonics and in May 1978 Stamper *et al.* were able to report irrefutable confirmation of their earlier experiment showing megagauss fields (*Phys. Rev. Lett.* **40**, 1177; 1978) but there remained an apparent conflict with the absence of such fields in the micro-balloon target experiments of Attwood.

It was perhaps an illustration of the coming of age of the new SRC Central Laser Facility in England that Raven, Willi and Rumsby, having an experiment scheduled just a few weeks later than that at NRL were able to take advantage of the Raman shifting idea and further to improve the experiment by the addition of simultaneous interferometry. The latter enabled direct determination of the electron density distribution which must be known to unfold the magnetic field from the Faraday rotation angle θ since $\theta \propto \int B_n n_e dl$. They produced an elegant set of results which gave the most accurate field determinations to date. Moreover their results removed the remaining discrepancy by confirming Attwood's low field values on 50 µm spherical targets and Stamper *et al.*'s megagauss fields on plane targets.

The current state of the art may be summarised by saying that the megagauss toroidal fields and associated tens of kiloampere currents do occur. The primary source of the field is known and in simple terms involves an imbalance in the diffusive flow of electrons in the plasma creating a current loop (Figure 1). The axial increase of electron number density N_e leads to an axial outflow of electrons and the radial decrease of T_e leads to a radial fall-off in this outflow. Charge conservation requires a current loop as illustrated and this is a thermoelectric type of generator creates a field increasing with time with

$$\frac{dE}{dt} \propto \nabla T_e \wedge \nabla n_e$$

and having a toroidal geometry as seen experimentally. The magnitude of the field is such that it has a significant pressure relative to the plasma (45% in the data of Raven *et al.*) and has a strong effect on electron diffusion by creating an electron cyclotron orbit diameter considerably smaller than the field free 90° deflection mean free path (7% in the data of Raven *et al.*).

In conclusion it is of interest to consider areas requiring further study. The relative importance of several other processes producing the magnetic field in addition to $\nabla T_e \wedge \nabla n_e$ is not yet fully clear. The effect of the field on heat flow

M. H. Key is in the Laser Division at the Science Research Council Rutherford Laboratory.

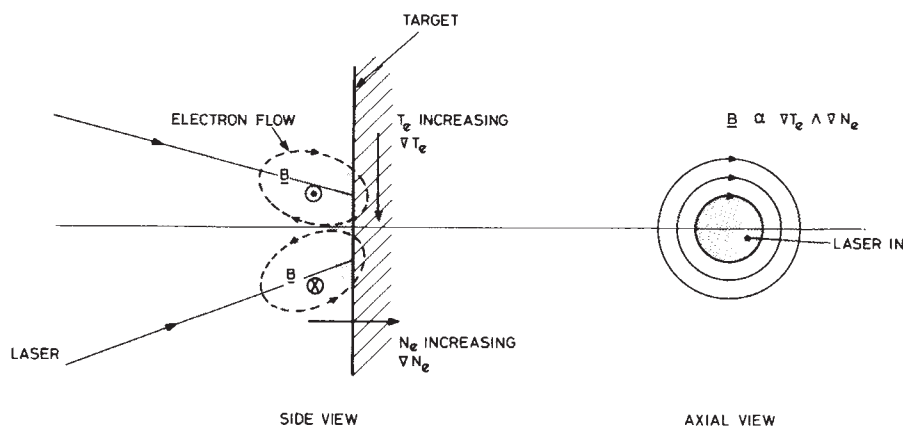


Fig. 1 Schematic diagram showing the target laser beam current path and magnetic field.

is also uncertain, first at the theoretical level as discussed by Max *et al.* (*Phys. Fluids* **21**, 128; 1978) and second because no experimental data have

been obtained on the field strengths in the region of a plasma of above critical density where heat transport by electrons is the dominant process. □

Calcium control of enzyme reactions

from Michael J. Geisow

THE diverse roles attributed to calcium ion as a mediator of biological control have proliferated to such an extent, that the symbolic representation of this element at the focus of schematic cells has become a common sight in reviews. Many intracellular calcium-binding structures, membrane complexes as well as discrete proteins, have been reported. Which of these can be considered as sites of calcium-mediated control? The normal ionised calcium level in the cytosol of cells is low (0.1 to 0.01 μM). Even under conditions of 'activation' like the response to a hormone, calcium ion concentration probably does not rise much above 10 μM . Moreover, this increased concentration is not uniform because of diffusional barriers in the cytosol, and it is transient because of the action of membrane calcium pumps.

Subcellular structures which are potential targets of calcium dependent regulation, must consequently have dissociation constants for calcium of the order of 1 μM . Additionally, since intracellular magnesium concentration is relatively constant at 1 mM, calcium binding sites must have a selectivity of at least 10^3 for calcium over magnesium. As far as is known, only proteins can meet these calcium-binding requirements.

The most universally accepted protein effector of calcium-mediated control is troponin C (TnC), which

makes muscle actomyosin ATPase sensitive to free calcium above about 1 μM (see *News and Views* **275**, 177; 1978). TnC has four divalent cation binding sites, of which two can only bind calcium. The protein molecule responds to bound cations with a large conformational change. TnC could be called the archetypal calcium receptor protein, since once actomyosin was recognised in non-muscle cells, the presence of proteins analogous in function to TnC was also anticipated. Kuo and Coffee (*J. biol. Chem.* **251**, 1603; 1976) isolated just such a protein from adrenal glands. Physically and chemically similar to TnC, this molecule bound two calcium ions with an apparent dissociation constant of 20 μM . This resulted in a considerable conformational transition, analogous to that undergone by TnC. Although it seems probable that this protein could activate actomyosin, the actual process regulated by the adrenal TnC remains undetermined.

By contrast, a variety of functions have been attributed to another intracellular calcium-binding protein, first identified in brain. This molecule, known as the calcium-dependent regulator or modulator protein (MP) has been purified from a wide variety of tissues. Although MP has chemical and structural homology with TnC, it is quite distinct and is two orders of magnitude less effective in stimulating actomyosin ATPase. At 1 mM Mg^{2+} , calcium ions bind to MP with an apparent dissociation constant of 3 μM

(Wolff *et al.* in *Calcium Binding Proteins and Calcium Function*, 97, North-Holland New York; 1977). The modulator protein activates cyclic nucleotide phosphodiesterase, adenylate cyclase and red blood cell Ca^{2+} -dependent ATPase (Parett & Penniston *J. biol. Chem.* **253**, 4676; 1978). Both MP and TnC inhibit and reverse microtubule assembly in the presence of 10 μM Ca^{2+} (Marcum *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3771; 1978). Indeed MP was localised by immunofluorescent labelling in the microtubule-rich mitotic spindle apparatus, implying its participation in the control of mitosis.

Many workers have found that the phosphorylation of membrane proteins is calcium sensitive. For example, Kruger *et al.* (*J. biol. Chem.* **252**, 2764; 1977) demonstrated that depolarisation of intact nerve-endings stimulated specific membrane protein phosphorylation, provided that this stimulus was delivered in a calcium-containing medium. Similar covalent phosphate incorporation occurred during histamine release from most cells (Sieghart *et al. Nature* **275**, 329; 1978). Recently Schulman and Greengard (*Nature* **271**, 478; 1978) showed that the phosphorylation of isolated nerve-ending membrane occurred in response to calcium and soluble cytoplasmic 'factors'. Purified MP was able to substitute effectively for the cytoplasmic proteins in the phosphorylation reaction. A common feature of the apparently diverse functions of MP is its connection with phosphate ester breakdown or synthesis. The essential link between all of the observations may have been provided by the identification of MP as a subunit of two protein kinases. Cohen *et al.* (*FEBS Lett.* **92**, 287; 1978) and Barylko *et al.* (*FEBS Lett.* **90**, 301; 1978) report that MP is a subunit of muscle phosphorylase kinase and myosin light chain kinase respectively. These kinases are sensitive to 1 μM Ca^{2+} , and it is likely that regulation occurs through the interaction of the intrinsic MP with a regulatory subunit of the kinases.

The modulation of cyclic nucleotide phosphodiesterase, adenylate cyclase and membrane Ca^{2+} -stimulated ATPases also seems to occur as the result of reversible binding of a calcium-MP complex to a regulatory subunit of these enzymes. It is interesting to speculate whether the regulation of microtubule polymerisation might result from an interaction of MP with tubulin GTPases (see David-Pfeuty *et al. Nature* **272**, 282; 1978.)

Although the status of TnC and MP as calcium-dependent regulators is now established, the way in which non-muscle cells could become activated as

Michael J. Geisow is a member of staff at the National Institute for Medical Research, London.

a result of contraction or phosphorylation/dephosphorylation processes remains obscure. In secretory tissues the universal response to raised cytosolic calcium is the release of secretory products stored in subcellular vesicles by fusion of vesicle and plasma membrane. Creutz *et al.* (*J. biol. Chem.* **253**, 2858; 1978) report the isolation of a 47,000 molecular weight protein which promotes the close association of adrenalin-containing granules isolated from adrenal glands. Contacts resembling the apposition of secretory granule membrane with plasma membrane before fusion, form reversibly in the presence of calcium ions above about 10 μ M. It was not established whether the granule-aggregating protein itself bound calcium, or became bound as a result of a calcium binding event on the granule itself. The latter alternative is more consistent with the observations of Gratzl *et al.* (*Biophys. biochim. Acta.* **470**, 45; 1977). These authors claim that aggregation and fusion of isolated secretory granules from several tissues occurs in the presence of 10 μ M calcium. Magnesium ions could not substitute for calcium in this process. A protein, rather than a lipid calcium-binding component, is implicated, since liposomes made from the lipids found in secretory granules do not aggregate or fuse at such low calcium concentrations.

The ability of the TnC-like proteins to confer calcium sensitivity upon enzymic processes, perhaps by combining with a common regulatory protein subunit, potentially accounts for the multiple intracellular effects of calcium. However, it is still premature to claim the uniqueness of these proteins as calcium receptors in non-muscle cells, and the characterisation of other recently identified members of this class of calcium-binding proteins is eagerly awaited. $\square$

The enigma of poxviruses

from Arie Zuckerman and Charles Rondle

THE global eradication of smallpox is probably the greatest public health achievement of the 20th century. Eradication was possible because of the availability of a highly effective vaccine prepared from vaccinia virus. Yet one of the most controversial aspects of vaccination is the origin of vaccinia virus, and in particular whether it is derived from smallpox or cowpox. It has also been suggested that vaccinia might have arisen as a hybrid between these viruses. It appears, however, that vaccinia virus is most closely related to cowpox. Phylogenetically, the whole group probably derives from one common ancestor and its differentiation must be connected with the domestication of animals by the first agricultural settlers of the human race.

The second reason for the success of the smallpox eradication programme is that smallpox is a human disease with no known animal reservoir or insect vector. But nevertheless, problems remain since natural poxvirus infection of nonhuman primates does occur (see **241**, 425; 1973).

Monkeypox virus was first identified in 1958 during an outbreak of a vesicular skin disease in captive *Cynomolgus* monkeys in Copenhagen (von Magnus *et al.*, *Acta Path.*

microbiol. scand. **46**, 156; 1959). The distribution of the rash was similar to that of smallpox in man and the virus was recognised as a poxvirus by its structural features under the electron microscope and by its serological relationship to vaccinia virus. By 1969, monkeypox virus had been isolated from captive nonhuman primates, or from their organs, on 10 occasions, including one isolation from an ant-eater which temporarily shared a cage with an infected orang-utang. Between 1970 and the middle of 1978, human cases of monkeypox were reported from West and Central Africa with five (15%) deaths, a mortality rate similar to smallpox in that region. Furthermore, most cases clinically resembled smallpox. The prospect of the spread of monkeypox from man to man was alarming, but in the event possible secondary transmission only occurred in two cases—of 55 susceptible family contacts only 2 (3.6%) acquired the infection, and there have been only 33 recognised cases among over 200 million people in a period of more than 8 years. But the natural host and vector of monkeypox virus remain unknown, despite the fact that there are reports of 'smallpox' in monkeys as long ago as 1767 (Arita & Henderson *Bull. Wld Hlth Org.* **39**, 277; 1968).

Whitepox virus is a more worrying puzzle. This virus was first identified in a Dutch laboratory in September 1964 during routine processing of Asian

Cynomolgus kidney tissues (Gispen & Brand-Saathof, *Bull. Wld Hlth Org.* **46**, 585; 1972) and could not be distinguished at the time from smallpox virus by laboratory tests. The name 'whitepox' was given to the isolates because in contrast to monkeypox they produced small dense white pocks on the chorioallantoic membrane of the developing chick embryo. Between 1971 and 1973, this virus was isolated on four occasions from primate kidney tissues derived from a chimpanzee and a sala monkey, and from two rodents, *Mastomys natalensis* and a squirrel-like rodent captured in Zaïre (Marennikova *et al. Bull. Wld Hlth Org.* **46**, 61; 1972; *Acta Virol.* **20**, 80; 1976). Experimentally, a whitepox virus inoculated into *Ceropithecus aethiops* monkey caused a generalised rash and illness. Whitepox virus still cannot be distinguished from smallpox virus by laboratory tests (Fenner *Prog. med. Virol.* **23**, 1; 1977), and it therefore remains both a puzzle and a threat. The fact that no infections with smallpox-like virus have occurred in Zaïre during the 7 years since the last smallpox case was identified is at least reassuring. It does seem, therefore, that animal poxviruses are not a threat to the global eradication of smallpox, but the need for constant surveillance and laboratory studies continues.

Marennikova and Shelukhina (see this issue of *Nature*, page 291) now report that whitepox viruses isolated



A hundred years ago

THE *Daily News* Quebec correspondent telegraphs as follows:—"After long study and many experiments Prof. Bell has made an important discovery in connection with the telephone. It is well known that the telephone has been a comparative failure in England on account of the fatal induction generated by the contiguity of other wires. Prof. Bell has discovered simple and efficacious means whereby not only is induction prevented, but the clearness and force of the telephonic vocalisation greatly increased. Prof. Bell tells me that practical demonstration of the importance of the discovery will be given in London as soon as the necessary preliminaries are complete." We give this statement as published in in the *Daily News*, though it must be received with some caution.

From *Nature* **19**, 14 November, 46; 1878.

Arie Zuckerman is Professor and Head of the Department of Medical Microbiology and Charles Rondle is Senior Lecturer, at the London School of Hygiene and Tropical Medicine.

from apparently healthy monkeys and rodents are stable clones which are present in populations of monkeypox virus. Inoculation of hamsters with monkeypox virus resulted in asymptomatic infection but virus was isolated from the hamsters for up to 7 weeks after inoculation. Four out of the 35 isolates showed that these were stable viruses identical with natural whitepox and which could not be differentiated from smallpox. It seems likely that the white clones were present in the original inoculum and were preferentially selected. This selection occurs in an unpredictable manner. Since whitepox viruses cannot be distinguished from smallpox virus, the results may be interpreted as an indication of the possible origin of smallpox viruses from monkeypox. □

What is climatology?

from T. M. L. Wigley and
P. Brimblecombe

Just what is the meaning of climatology as a discipline, and what qualifications or interests does one have to have in order to contribute to climatology? The recent conference 'Evolution of Planetary Atmospheres and Climatology of the Earth' in Nice* emphasised, perhaps more strongly than any similar gathering, the range of fields which now contribute to our understanding of past climates. Very few of the participants could call themselves climatologists in the traditional sense of the word; they came variously from astronomy, geology, space physics, geochemistry, statistics, glaciology, oceanography, botany/palynology and other areas of science.

The conference itself had very ambitious aims. There were papers dealing with climatic change on all time-scales, from about 10^8 years down to seasonal changes, beginning with a review of the longest time-scale changes on Earth, Mars and Venus by T. Owen (State University of New York, Stony Brook). He pointed out some of the problems with the assumption that the Earth originally had a highly reducing atmosphere (NH_3 , CH_4), and stated the present general view that CO_2 and H_2O were important original constituents. This hypothesis was strongly supported by A. Henderson-Sellers (University of Liverpool) and A. J. Meadows (University of Leicester) who gave results from a model which, on the basis of a CO_2 - H_2O atmosphere, was able to explain the so-called 'faint Sun paradox': that is, the apparently

anomalous lack of extensive ice cover on the Earth during its early history when the solar output was substantially less than today. A number of other papers dealt with this contentious issue, an issue that has ramifications for the origin of life on Earth. Most experimental synthesis of the prebiotic formation of organic molecules begin with a strongly reducing atmosphere, but this and other recent work suggest that such a beginning is not a prerequisite for the evolution of life.

Bridging these and shorter time-scale studies was the study of mid-Cretaceous global climate presented by P. Larsson (University of Lancaster) which suggested that dinosaurs may well have been warm-blooded animals. On the 10^6 to 10^7 -year time-scale, N. J. Shackleton (University of Cambridge) presented stimulating evidence on Tertiary climatic change which is at least as challenging to the theoretician as the faint Sun paradox. In the mid-Tertiary, for a period of $\sim 10^7$ years, it seems that tropical and subtropical climates were some 4 to 6 °C cooler than today: that is, colder than during any of the 17 or so glacial cycles which have occurred in the past 2.5×10^6 years. For the climatologist, such a climate picture is, at present, very hard to understand. It lies outside the possible realm of Milankovitch effects which are now generally accepted as being fundamental to the more recent glacial cycles. The Milankovitch theory was, however, treated with healthy scepticism in W. Broecker's review paper (Columbia University). The paper by A. Berger (Louvain University, Belgium) indicated that monthly insolation data may be more important in determining the climate-orbit link than the data used by Broecker, but Broecker's suggestion that there may still be important internal mechanisms was reinforced by some interesting modelling experiments reported by M. Ghil (New York University) and K. Bhattacharya (Columbia University). Their work, though based on very simple models, produced purely internal cyclic variations with nearly square-wave behaviour. They impose an imaginative albedo-temperature relation to obtain this cyclic solution. The amplitude of the cycles is reasonably in accord with past evidence, a particularly interesting factor since the modelling experiments based on external forcing (*à la* Milankovitch) invariably fail to obtain a climate signal with realistic amplitude. These models were represented by G. R. North (University of Missouri) and J. A. Coakley's (National Center for Atmospheric Research, Boulder) elegantly simple seasonal model, and by the models discussed by E. Bernard (Institut Royal

Météorologique, Brussels) and by C. Nicolis (Institute d'Aéronomie Spatiale, Brussels) (although the latter's efforts were directed towards longer time-scale climatic fluctuations), and, in a later session by the work of M. Hantel (Bonn University).

The Milankovitch theory was once again strongly supported in the paper by J. D. Hays and D. W. Cooke (Lamont-Doherty Geological Observatory, Columbia University). They showed that major climatic fluctuations were approximately in phase between Northern and Southern hemispheres over the past 5×10^5 yr, and that the well-established periods of 23,000, 41,000 and 100,000 yr also show up in Antarctic deep-sea cores. These authors also suggested that the glacial to interglacial transition, as evidenced by a reduction (by a factor of ~ 10) in Antarctic summer sea ice, might have occurred in as little as a few hundred years, and that such changes preceded major ice volume changes in the Northern hemisphere.

Interesting papers on 3×10^6 and 3×10^4 -yr time-scales were presented by J. C. Duplessy (Centre des Faibles Radioactivités, Gif-sur-Yvette) and C. Lorius (Laboratoire de Glaciologie, Grenoble), but the latter part of the conference was devoted to a much shorter time-scale. A brief review by T. M. L. Wigley (University of East Anglia) pointed out that the European Medieval warm epoch (approximately AD 1000–1200) was probably not a global phenomenon, in contrast to the apparent ubiquity of the climax of the Little Ice Age (AD 1500–1700). This picture provided a backdrop to a number of detailed descriptions of climatic events during the past 1000 years, descriptions which were highlighted by the interesting dendro-climatological studies of F. H. Schweingruber, O. U. Bräcker and E. Schar (Swiss Federal Institute of Forestry Research, Birmensdorf), A. Berger and co-workers (Louvain University), and F. Serre (Marseille University). Schweingruber's group are pioneers in using wood density as a proxy climate indicator (closely related to summer temperature), and these workers showed some intriguing evidence of teleconnections between North America and Europe on the 5-year time-scale.

One of the most immediately significant contributions came from C. E. Leith (National Center for Atmospheric Research, Boulder, Colorado) on the mechanisms of short period climatic fluctuations. He gave a most lucid explanation of predictability, based largely on the work of Madden. If, as

T. M. L. Wigley and P. Brimblecombe are in the Climatic Research Unit at the University of East Anglia.

*Held 16–20 October, 1978.

is so in the present state of the 'art', weather is essentially unpredictable beyond about a 5-day period, then the statistics of observed climate variability imply, necessarily, that month to month climate changes are, for many parts of the world, largely unpredictable. Although this is an undisputable statement of present capability, there are some rays of hope sneaking over the horizon. For instance, there is some indication that, if we knew more about the mechanisms of 'blocking', then there would be greatly improved predictability in these situations. Furthermore, as S. H. Schneider (Boulder, Colorado) pointed out, we may still be able to develop good selective prediction schemes (for extreme events, for example) even without improving day to day forecasting skills or our understanding of the meteorology of blocking.

Schneider himself presented one of the most interesting review papers, on the effects of man's activities on climate. Although man's effects, on the regional and global scale, are not yet distinguishable from 'natural' climatic fluctuations, the potential for more significant effects is high. Because of the immobility of society *en masse* we are, in most places, unable to adapt rapidly to changes in the climate of marginal regions (be these changes natural or man-made), so possible man-induced changes are of considerable concern. In particular, the magnitude of CO₂ effects before the middle of next century could be 1 or 2 °C overall at the surface, and much greater in polar regions. This would have serious consequences. This point was reinforced in an engrossing summary given by Professor H. Flohn (Bonn University). He considers two factors of prime future importance. First, in the past there is strong evidence of extremely rapid climatic change—as fast as, for example, a 5 °C drop in summer temperature in less than 100 years. Should this happen in the future, there could be dramatic consequences. Second, the production of CO₂ by fossil fuel burning is liable to be a major problem within 100 years. On a reassuring note, however, Flohn pointed out that even a large warming would probably not, as some people have suggested, cause a rapid melting of the Arctic sea ice. Partly because of feedback effects and partly due to the large time constant for the Greenland Ice Sheet the minimum time for such melting would be at least 1000 years. And, although this is certainly not a cause for complacency, we are still not absolutely certain that increased CO₂ will lead to warming. All models used to date to examine this question have failed to handle possible cloudiness changes in a realistic way: we just do not know

how to do so at present. Still, the various models, many of which parameterise clouds very differently, all predict warming, so the odds are strongly in favour of such an outcome and it is a possibility which must be taken with utmost seriousness.

Although many significant advances have been made in climatology in recent years, it is sobering to consider these in the light of the first climate model; that of the Egyptian scribe who said, around 3000 BC, "the Sun warms the Earth". □

Histone genes displayed

from P. J. Ford

HISTONE proteins are of central importance to the molecular biology of higher organisms since with DNA they are involved in chromatin structure and function. The five types of histone, H1, H2A, H2B, H3 and H4 are found combined with an equal amount by weight of DNA in nuclei of eukaryote cells. The relative amount of each histone type appears to be regulated since they are present in equal molar amounts, except H1 at half molar amount. In addition there are in many organisms developmental stage and tissue-specific sequence variants within each of the major histone groups. Quite how these variants relate to chromatin structure and function is not clear, but they provide a useful handle for studies on the control of specific gene expression during development and differentiation. As is often the case recent advances in understanding histone gene structure stem from the discovery of a particularly favourable system, early sea urchin development, in which rapid cell division demands considerable rates of histone synthesis to accommodate the high rates of DNA synthesis, and from which histone mRNA is readily extracted (Kedes & Gross *Nature* **223**, 135; 1969). Estimates of histone gene numbers in sea urchins suggested the presence of several hundred to a thousand copies of each histone gene depending on species (Kedes & Birnstiel *Nature new Biol.* **230**, 165; 1971). In this respect sea urchins may be an extreme case since other phylogenetically diverse groups have considerably fewer (20-100) copies of each histone gene.

Isolation by molecular cloning techniques of DNA segments coding for sea urchin histone genes has led to proof that genes for all five histone

types occur together in one repeating unit in the order H4, H2B, H3, H2A and H1 and that each structural gene is separated from the next within the unit by spacer DNA. Recently (Schaffner *et al. Cell* **14**, 655; 1978) Birnstiel and his colleagues have presented DNA sequences for more than half the total histone gene repeat unit known as h22 from the sea urchin *Psammechinus miliaris*. These include complete sequences for the H3 and H2A genes plus almost complete sequences for the other three structural genes and entirely confirms earlier maps of this particular DNA fragment. From a comparison of known histone protein sequences with the amino acid sequences which would be generated by translation of these particular structural genes it seems likely that unit h22 is active in early development and does not code for the specific histone subtypes found in sea urchin sperm. Additional information derived from these sequences is that h22 histone coding sequences do not contain inserted untranslated sequences like those seen in mouse and rabbit globin and chicken ovalbumin genes. Histones are not synthesised as longer precursors and there is no evidence for substantial regions which might be translated in any other reading frame to give other types of polypeptide.

As well as sequences for structural genes Schaffner *et al.* have determined sequences for regions at the beginning and end, called prelude and trailing sequences respectively, of the structural genes and for some of the spacer regions. As they state: "If it were true that identical functioning of the regulatory signals require exactly identical primary structures, then sequence comparisons between preludes and trailing sequences around different histone genes should reveal some sequence homologies. Our sequence comparison by computer analysing runs of three or more nucleotides has been relatively unsuccessful in that it has disclosed no long runs of sequences held in common between different spacers or preludes, with the possible exception of a short sequence in two spacer regions and a sequence of twenty nucleotides in the prelude regions of the H2A and H4 structural genes". I suppose we are to conclude that either the sequences presented do not cover regulatory sites or that they function in a way which does not require extensive sequence identity.

Sequences and maps of restriction enzyme sites in spacer DNA of h22 has revealed that unlike the spacers found in *Xenopus* ribosomal and 5S RNA genes there are no small repetitious sequences present. Consequently no spacer length variation like that in the

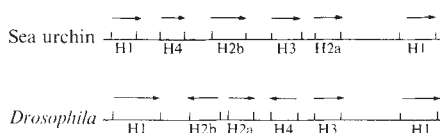
Peter J. Ford is in the Department of Molecular Biology, University of Edinburgh.

Xenopus rRNA genes due to the presence of different numbers of small repeating units is observed in sea urchin histone DNA. This is not to say that length variation does not occur, since Overton and Weinberg (*Cell* **14**, 247; 1978) studying independently isolated histone gene units from another sea urchin *Strongylocentrotus purpuratus* report that there is both length and sequence variation between different histone gene units. They have been able to show that this variation is found more extensively in histone DNA isolated from a group of sea urchins. More remarkable is that histone DNA variants from individual sea urchins are limited to a few of the major classes seen in the population as a whole. Each of five individuals were distinguishable on the basis of histone gene length and restriction site variants. Before attempting to assess what this means it will be necessary to follow histone gene patterns in offspring of individuals of known histone genotype.

During early sea urchin development two sets of histone genes are active. Kunkel and Weinberg (*Cell* **14**, 313; 1978) provide evidence that a full length histone DNA repeat, called pCO1, from *S. purpuratus* will hybridise to histone RNA from both cleavage and blastula stages. The hybrids formed with blastula transcripts are more easily melted (10 °C) and more easily digested with ribonuclease suggesting more extensive base mismatching than hybrids with cleavage stage transcripts and hence blastula transcripts probably derive from another type of histone gene. They suggest that pCO1 is preferentially transcribed during cleavage and not during blastula when transcription of other histone genes predominates. Such changes in transcription are also reflected by changes in histone protein synthesis (Newrock *et al.* *Cell* **14**, 327; 1978). Changes in the *in vivo* patterns of histone synthesis during early sea urchin development may be transferred to an *in vitro* protein synthesising system along with mRNA isolated from the appropriate developmental stages. There is no evidence from these studies for control of histone subtype synthesis at the translational level, since new subtype synthesis *in vivo* is closely coupled to appearance of mRNAs which can be translated *in vitro* to give products with very similar properties to the new histone subtypes seen *in vivo*.

Recently (Lifton *et al.* *Cold Spring Harbor Symp. quant. Biol.* **42**, 1047; 1977) the structure of *Drosophila melanogaster* histone genes has been reported. There are some interesting differences as well as similarities in

comparison with sea urchin genes. Both contain all five histone coding sequences together in a single repeating unit, but the order and the direction of



transcription differs. As the promoters for transcription have not been identified yet it is not clear whether each structural gene has its own promoter or whether there is a single promoter for each repeat unit, the resulting polycistronic primary transcripts being processed to give separate mRNAs. In *Drosophila* the fact that transcription must proceed in both directions makes a single promoter for both strands very unlikely. Lifton *et al.* have made an unsuccessful search for high molecular weight RNA molecules which hybridise to histone DNA and that might be considered polycistronic transcripts. They consider it most likely that *Drosophila* histone genes are transcribed independently, possibly from two divergent promoters and a single promoter. In sea urchins it is still unclear where transcription is initiated. The fact that clustering of different histone structural genes together in one unit is a very old and evolutionarily stable organisation suggests that it might be important perhaps in allowing the co-ordinated transcription of equal numbers of genes, thus ensuring correct histone protein stoichiometry, providing all histone messages are translated equally efficiently. □

A threat from fluorocarbons?

from a Correspondent

Do chlorofluorocarbons (CFCs) represent a threat to the ozone layer, with possible implications for life on this planet? This question, prompted by the hypothesis of Molina and Rowland (*Nature* **245**, 810; 1974), was addressed at 'Fluostrat '78'. According to Molina and Rowland the breakdown in the stratosphere of CFCs, whose industrial production mushroomed in the early 1970s, would produce Cl and ClO which would reduce ozone through a catalytic cycle.

The representatives of the chemical industry went to great pains to point out the very large amount of work on

this problem which has been sponsored by the Manufacturing Chemists Association. They pointed to the range of qualities which has made the CFCs such ideal propellants. The search for alternatives has, so far, produced no compounds with even comparable properties. However, the case to be answered remains whether or not CFCs could reduce the amount of ozone in the atmosphere, thus allowing increased penetration of potentially harmful ultraviolet radiation.

The scientific proceedings of the symposium suggest that we are still some way from a definite answer to this question. Indeed, the interesting feature to emerge was the increasing awareness of the uncertainties in all the areas of research and of the great complexity of the system which atmospheric scientists are attempting to study.

A particularly important area of research is the investigation of the levels of CFCs present in the troposphere, in an attempt to determine their lifetimes there. J. E. Lovelock dealt with the difficulties in calibrating the electron capture gas chromatographs used in the measurement of CFCs. In a very elegant process, he has used an exponential dilution chamber to dilute accurately weighed amounts of CFCs to the parts per trillion (10^{-12}) level and then tested the calibration assumptions made by most experimenters. These assumptions have been found by Lovelock to be invalid over the range of mixing ratios measured in the atmosphere. Indeed, an intercomparison of a prepared standard sample of CF_2Cl_2 by 12 different laboratories showed very large differences between their findings.

It is apparent that the natural cycle of chlorine compounds in the atmosphere is not well understood. There was, in particular, much energetic debate regarding the apparently high levels of stratospheric ClO which have been reported by Anderson *et al.* (*Report to Congress and to the Administration of the Environmental Protection Agency on the National Aeronautics and Space Administration*, 1978).

B. A. Thrush of Cambridge University, in reviewing atmospheric chemistry, dealt with the importance of the coupling between the cycles of the nitrogen and chlorine compounds. The theme of the importance of meteorological coupling mechanisms was taken further by R. J. Murgatroyd (Meteorological Office, Bracknell) and J. A. Pyle (Oxford University). The numerical models which have been used in predictive studies have generally been rather simple, one-dimensional (height only) representations of the atmosphere. The need for models which represent meteorological processes rather better is apparent and these

*Held in Brighton, UK on 5-6 October, and organised by the Society of Chemical Industry.

models should include the important coupling between chemistry, radiation and dynamics. Model calculations of ozone depletion were shown to be sensitive to these feedback mechanisms.

The problem of just what effect an increase in ultraviolet radiation might have on the population was discussed by R. H. Mole of the Medical Research Council Radiobiology Unit, Harwell. He found faults with a wholly uncritical acceptance of the correlation between latitude and occurrence of skin cancer. Changing social conventions in dress and increasing mobility of the population are certainly likely to be very important factors.

In summary, the symposium in reviewing recent work highlighted many uncertainties. There is clearly a need for a better understanding of the chlorine budget, the continuing measurement programme being very important. Further improvements in numerical models are necessary, with greater emphasis on the meteorology of the problem. Finally, it would be of great help to the planners who must make some decision on the use of CFCs if a more reliable understanding of the possible medical effects of an increase in ultraviolet radiation dosages were gained. □

Microscopic optical potentials

from P. E. Hodgson

AN international workshop on microscopic nuclear optical potentials was held at the University of Hamburg from 25 to 27 September.* Organised by Professor H. V. von Geramb, it attracted over 100 participants and provided a welcome opportunity to review the recent progress in calculating optical potentials from the nucleon-nucleon interaction. Powerful methods of calculating the optical potential for finite nuclei have been developed by Jeukenne, Lejeune and Mahaux in Liège and by Rook and Brieva in Oxford (*Nature* **253**, 163; 1975 and **269**, 750; 1977). The resulting potentials fit the data quite well, without further parameter adjustment. This opens the way to a detailed understanding of the interaction of nucleons and groups of nucleons with nuclei, assists the phenomenological analyses of experimental data, and should stimulate further accurate experimental work. The latest developments in these calculations

were reviewed by C. Mahaux and by F. A. Brieva, and related work by I. McCarthy (Adelaide) and M. Weigel (Munich).

At lower energies, the structure of the target nucleus affects the interaction, and the effects of the coupling to the single-particle and collective states may be included in the calculation of the imaginary part of the potential by explicit summation over intermediate states. Work of this type was described by V. A. Madsen (Oregon), N. Vinh Mau (Orsay) and Van Giai (Orsay), and applied to nucleon and alpha-particle scattering from ^{40}Ca and ^{208}Pb .

It is now clear that the Saxon-Woods form factor universally used in optical model calculations is not always a sufficiently accurate representation of the potential. Improvements can be made by using microscopic form factors, or by supplementing the potential by an extra term depending on the orbital angular momentum L , or by altering the shape of the potential in a systematic way to fit the experimental data. A microscopic basis for an improved potential was provided by Y. C. Tang (Minnesota) who used the resonating group formalism to show that antisymmetrising the wavefunctions in a folding model calculation gives rise to parity-dependent terms in the potential. These take account of exchange processes and become less important as the mass difference between the interacting particles increases.

In some interactions there is a significant difference between the optical model predictions and the experimental data, and there is an obvious physical reason. It is then possible either to incorporate the physical effect in the calculations, or to add an extra phenomenological term to the potential. An example of this is the strong coupling to low-lying collective states which in the case of sub-Coulomb scattering of heavy ions causes the cross section to depart from the usual Fresnel form. This was discussed by A. J. Baltz (Brookhaven), who showed that the data can be fitted by the addition of a long-range dynamic absorption potential that can be obtained in closed form. Further analysis of this process was presented by G. Baur (Jülich). Another example is provided by the strong coupling to particular reaction channels. This alters the elastic cross section in a way that can be fitted by an L -dependent potential, as shown by Mackintosh (Daresbury). He also showed the value of model-independent analyses of elastic scattering.

Extensive work on the unification of the bound state and scattering potentials was presented by M. M. Giannini (Genova), and other global phenomenological analyses by A. Tarrats (Saclay) and by H. Leeb (Vienna). A

new method of measuring cross section ratios was described by S. M. Austin (Michigan) and extensive measurements of proton scattering by light nuclei by M. Pignatelli (Milano).

The analysis of the scattering of composite particles by nuclei has not yet been developed to the same stage of sophistication as nucleon scattering. Several folding models give semi-phenomenological potentials that often fit the data quite well, but much of the important physics, especially the effects of antisymmetrisation and exchange, is still hidden in the adjustable normalisation parameter. A pioneer study of deuteron interactions was presented by A. A. Ionnides (Surrey) but apart from this the development of microscopic potentials for composite particles is still in the future. Meanwhile, a wealth of accurate data on the elastic scattering of deuterons and alpha-particles was presented by several groups, and in most cases they are very well fitted by optical model analyses.

Calculations of the imaginary part of the optical potential for heavy ions were reviewed by D. M. Brink (Oxford) who also described a new approach using the Feynmann path integral method. This can be used to relate the imaginary potential to the friction coefficient in heavy-ion scattering. D. F. Jackson (Surrey) showed how the extreme sensitivity of the alpha-decay rate to the potential barrier can be used to define the optical potential more precisely. A repulsive-core alpha-particle potential that gives the energies and widths of unbound cluster states and also fits the elastic scattering was described by K. A. Gridnev (Leningrad). Molecular states in heavy ion potentials were reviewed by W. Scheid (Giessen).

A fully microscopic description of heavy-ion reactions is not yet practicable, but considerable progress has been made using the time-dependent Hartree-Fock method. P. G. Reinhard (Mainz) has used this method to determine the collective Hamiltonian and hence the quantum corrections to the optical potential.

The meeting showed the extent and vitality of studies of all aspects of optical model potentials. The techniques for calculating microscopic potentials have now been established for nucleons, and much work remains to be done to improve the calculation of small terms and to apply the method to a wide range of nuclei and energies. At the same time it will be extended to composite particles, to link up with the very extensive phenomenological analyses that have already been made.

P. E. Hodgson is in the Department of Nuclear Physics at the University of Oxford.

*A summary of the workshop is available on request from P. E. Hodgson. The papers will be published by Springer Verlag as a volume in the Lecture Notes in Physics series.

review article

Advanced batteries

B. C. Tofield

Materials Physics Division, AERE Harwell

R. M. Dell

Materials Development Division, AERE Harwell, Oxfordshire, UK

J. Jensen

Department of Chemistry, Odense University, 5230 Odense, Denmark

Advanced-battery technology has been the basis of an Anglo-Danish interdisciplinary scientific project. The technological and economic aspects of advanced battery installations in vehicular and stationary applications are discussed in this article.

ADVANCED batteries provide a particularly good example of interdisciplinary science and technology. To gain the scientific understanding necessary to predict appropriate materials for constructing such devices and then technically to build a successful device, one must bring together such subjects as structural chemistry, solid- and liquid-state electrochemistry, interfacial and surface science, ceramics, corrosion science and metallurgy. Solid state physics and computer modelling of ionic motions within phases and across interfaces also have an important part to play. To assess the economic viability of particular systems one has to take account of projected battery performance parameters as well as such features as cost and availability of raw materials, processing technology, construction and fabrication costs, installation costs, safety, pollution and a comparison of these aspects weighed against alternative means of doing the same job.

An advanced battery project has recently started at several laboratories in the UK and Denmark. Seven institutions, four in England and three in Denmark, are collaborating to do research on materials for advanced batteries, together with technological and economic assessments of advanced battery installations in vehicular and stationary applications. The work is partially sponsored by the Commission of the European Communities.

β Alumina and the sodium-sulphur battery

Advanced batteries have stimulated much interesting work in solid-state chemistry and electrochemistry in recent years¹⁻³. The seminal discovery was the observation of very high sodium ion mobility above room temperature in sodium β alumina by

Weber and Kummer (at Ford) just over 10 yr ago⁴. β alumina, one of a family of closely related compounds, has a layer structure as shown in Fig. 1. Four layers of close-packed oxygen atoms contain four fold and six fold coordinated aluminium in an atomic arrangement analogous to that found in spinel, MgAl_2O_4 . The formula of β alumina, however, $\text{Na}_{1+x}\text{Al}_{11}\text{O}_{17+\frac{x}{2}}$ is not compatible with an infinitely extending spinel structure. The relative lack of aluminium is reflected in the fact that every fifth layer of oxygen atoms is only one-quarter filled. The sodium ions are found in this relatively empty layer⁵.

β Alumina was so named because it was thought to be an isomorph of aluminium oxide, and the crystal structure was essentially solved before the war⁶. However, the unusual electrical properties of sodium β alumina were not discovered until recently. When the electrical conductivity at 300 °C was measured, and it was realised that the current carriers were exclusively sodium ions and not electrons, the application of materials like β alumina to a modern generation of power batteries was quickly realised. In the sodium-sulphur battery, patented by Ford, instead of solid electrodes separated by a liquid electrolyte, as in the conventional lead-acid car battery, for example, sodium β alumina is used as a solid electrolyte, specifically conducting sodium ions, between liquid electrodes of sodium metal and sulphur (Fig. 2). The cell voltage, 2.08 V, is derived from the chemical reaction between sodium and sulphur to produce sodium polysulphide and the theoretical energy density, about 750 Wh kg⁻¹, is high compared with that of the lead-acid battery, only about 170 Wh kg⁻¹. Such impressive energy densities immediately indicated attractive possibilities for electric vehicle propulsion and other applications.

The novelty of the properties discovered in sodium β alumina may be estimated from the fact that, although its melting point is around 2,000 °C, several A cm⁻² of current may be passed across the electrolyte at 300 °C. The key to the rapid sodium ion mobility lies in the crystal structure and the low potential energy path for sodium migration this produces. β Alumina thus provides a happy hunting ground for molecular dynamics and potential energy calculations^{7,8} as well as for experimental

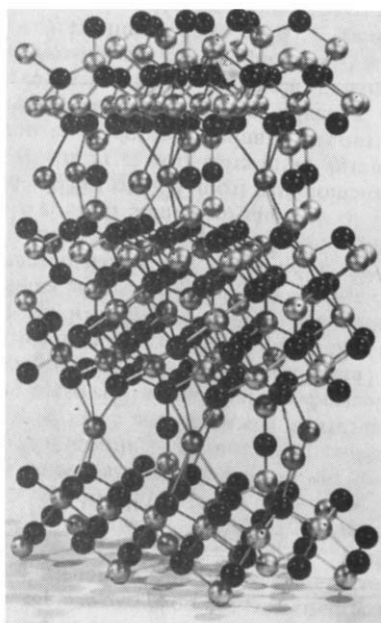


Fig. 1 The β alumina structure. The densely populated close-packed 'spinel blocks' containing aluminium and oxygen ions separate the mirror planes which contain the mobile sodium ions. The density of oxygen atoms in the mirror planes is only one-quarter of that in the spinel blocks. The separation between mirror planes is just over 11 Å.

assessment of the effect of chemical structure variations and ion exchange on the electrical behaviour^{9,10}. Many other cations including the other alkali metals and ammonium, protons and hydronium ions can be ion-exchanged for sodium by immersion in suitable molten salts or by other techniques¹¹.

Although the gross features of the β alumina structure have been known for a long time, the detailed crystallography of the mirror plane, vital to an understanding of the electrical properties, is still only partially understood. Recent work using neutron diffraction at Brookhaven National Laboratory has shown¹² that the occupation of the mirror plane is considerably more complicated than originally thought. Sodium β alumina as normally produced is invariably non-stoichiometric with x in the formula usually between 0.2 and 0.3, and it is now generally accepted that this non-stoichiometry is accommodated by extra oxygen as well as extra sodium occupying positions in the conducting plane. The actual conduction mechanism of sodium is, therefore, complex and has not yet been fully unravelled. Indeed, the mirror plane chemistry of the β alumina structure can almost be called a branch of science in its own right.

One interesting new development at Harwell has been the characterisation of hydrogen-conducting β aluminas. These have been studied electrochemically and gravimetrically^{13,14} and by neutron diffraction¹⁵. A proton-conducting ceramic could have interesting applications, for example, as a medium temperature fuel-cell electrolyte.

An added complication in β alumina chemistry is that several variants of β alumina exist⁹ and so-called β'' alumina, which has cubic rather than hexagonal stacking of the spinel blocks (as in β alumina) has a higher conductivity than β alumina but is more susceptible to attack by moisture and is not so rugged. A compromise between conductivity and strength may be obtained by having both β and β'' present in electrolyte tubes. High resolution electron microscopy has shown¹⁶ that the mixing can occur on the unit cell level as well as by a mixing of macroscopic grains. The intergrowth of β - and β'' -alumina layers is shown in Fig. 3. The higher conductivity of β'' alumina is related to the fact that the different stacking of the spinel blocks produces two equivalent cation sites in the mirror plane rather

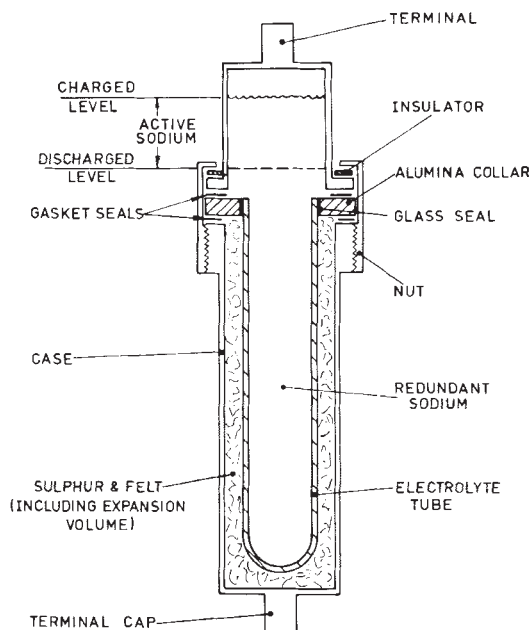


Fig. 2 A schematic model of the sodium-sulphur battery which uses a sodium β alumina solid electrolyte as the separator between liquid electrodes (sodium anode and sulphur cathode). The operating temperature is 300–400 °C.

than one favoured site and one relatively blocking site as in β alumina, and also to the absence of interstitial oxygen in the mirror plane¹². β'' alumina is non-stoichiometric with regard to sodium, an essential feature for rapid ionic motion, but this is compensated by magnesium substitution, $\text{Na}_{1+x}\text{Al}_{11-x}\text{Mg}_x\text{O}_{17}$ (with x usually between 0.2 and 1.0) or by lithium substitution rather than by interstitial oxygen.

The new science of rapid ion transport

The discovery of β alumina and the promise of the sodium-sulphur battery have had parallel effects in science and technology. Many programmes in several countries were set in motion to develop sodium-sulphur batteries¹⁷. Nevertheless, although development has been going on for 10 yr, batteries are not expected to be commercially available until the mid 1980s.

At the same time as the work on battery development, many scientific centres, particularly in the US and Europe, started programmes to try to discover new fast ion conducting solids, both for sodium, other alkali ions, and anions such as fluoride. For several years this search was not very successful, but as the structural requirements have become better understood alternative materials have begun to emerge (see, for example, refs

18–20). However, no sodium or other alkali ion-conducting materials have yet been found which show significantly improved electrical properties over β alumina and none has as low an activation energy for ionic mobility (0.13 eV) (ref. 21). Fast ion-conducting materials have been listed and discussed^{22,23}, and the field of solid state ionics and the technical applications, including advanced batteries, has recently been surveyed³⁰.

One new and important concept which has emerged, involving fast ion conductivity, is the insertion compound or solid-solution electrode²⁴. In a solid-solution electrode there is both rapid ion transport and electronic conductivity. But a third vital ingredient is the presence of a range of stoichiometry involving the mobile ion. Fast-ion conducting materials of this type, therefore, can be used, not as electrolytes as is the case for β alumina, but as battery electrodes with either a liquid electrolyte or a solid electrolyte. In the latter case one is using fast ion-conducting materials to make an all-solid-state battery. In the former case, although the arrangement of solid and liquid phases is formally similar to that found in conventional lead-acid or alkaline batteries, the power-to-weight ratios obtainable using modern materials are much higher than was previously thought possible.

In contrast to solid electrolytes, where sodium is generally found to be the most mobile of the alkali ions, most good solid-solution electrode materials so far discovered act best as conductors of lithium ions, although this distinction may not survive further research. One of the most attractive solid-solution electrodes so far reported²⁵ is based on titanium disulphide, TiS_2 . This is a layer material with adjacent layers of sulphur atoms held together only by van der Waals bonding (Fig. 4). TiS_2 and other similar chalcogenides of the transition metals can absorb alkaline cations between the sulphur layers. There is a slight expansion of the crystal axis perpendicular to the layers but otherwise no change in the crystal structure. For lithium going into titanium disulphide there is a continuous region of non-stoichiometry from TiS_2 to LiTiS_2 . Moreover, the partial free energy of intercalation is relatively constant across the whole range. Titanium disulphide is quite light, cheap and readily available and the lithium-titanium sulphide battery developed by Exxon²⁶, which has an organic electrolyte and works at room temperature, has a theoretical energy density of 480 Wh kg^{-1} . This is already quite close to that of the sodium-sulphur couple, and there is the advantage of room-temperature operation, the absence of liquid alkaline metals, and, possibly, considerably reduced problems in fabrication and corrosion.

Solid-solution electrodes therefore provide a field of complementary scientific interest to solid electrolytes and have at least equal potential application in battery systems. Apart from the presence of rapid ion transport, the extra keys to the utility of solid-solution electrodes lie in the interfacial aspects of ions being able to pass directly between the electrolyte phase and the electrode without any change in crystal structure of the electrode or the necessity to form a new compound by electrocrystallisation (compare the electrode reactions in lead-acid and alkaline batteries), and the ability of the electrode to absorb a sufficient quantity of the active ion to provide systems with very attractive energy densities. The use of metal chalcogenides as reversible electrodes has recently been reviewed²⁷.

A third, more traditional, concept being applied in advanced battery design is the application of fused-salt electrolytes. The use of fused salts, of course, implies elevated temperatures, but fused salt electrolytes can allow the drawing of higher current densities than is presently possible with solid electrolyte-based batteries. The most notable current development using a fused-salt electrolyte is the lithium-iron sulphide battery, developed at the Argonne National Laboratory²⁸. This battery operates at around 400°C and uses a fused salt eutectic electrolyte mixture of lithium chloride and potassium chloride. Using a lithium-aluminium anode, the theoretical energy density is 650 Wh kg^{-1} , similar to that of sodium-sulphur, and the expected actual energy densities of the two systems are comparable.

The need for advanced batteries

Battery development is, therefore, at an interesting stage of transition and, although estimates differ and there will obviously be wide variations from country to country, considerable applications for advanced batteries can be foreseen in the medium- and long-term future. Future electrical energy supply will almost certainly have an increasing contribution both from nuclear power and from alternative sources such as wind, waves and solar energy. Irrespective of the relative importance of these various sources, there will be an increasing need for energy storage capacity to match the demand to the supply characteristics. Although desirable, a radically improved standard of energy economy does not change such a need. There are several ways of achieving such matching but electrical storage by

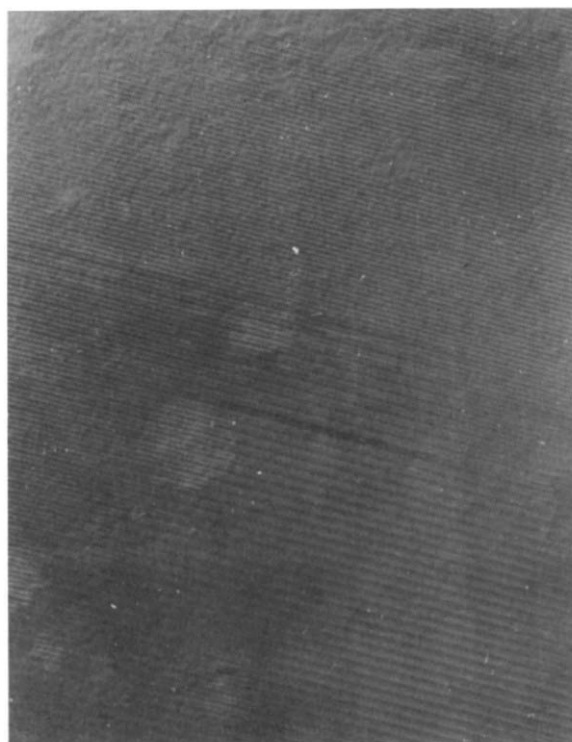


Fig. 3 A lattice image of a mixture of β alumina and β'' alumina showing intergrowth of the two structures at the unit cell level. The electron beam is parallel to the mirror plane. Although the spinel block thickness is the same in both materials, the space groups are different. Double diffraction is allowed in the $\text{P6}_3/\text{mmc}$ space group of β alumina and the 'forbidden' (001) reflections can be imaged in suitable conditions to generate $22 \text{ \AA}$ fringes. Only the space-group-allowed $11 \text{ \AA}$ spacing reflections are seen for β'' alumina.

secondary batteries offers one attractive method over a short timescale.

Additionally, efficient off-peak energy utilisation by relatively non-polluting electric vehicles will surely play an increasingly important role in the total picture²⁹, although for such a role to be significant it is particularly important that electric vehicle technology is developed to provide products which are economically competitive with internal-combustion-engine-powered vehicles. The extent to which this can be achieved depends critically on the development of cheap, long-lifetime, high-energy batteries capable of many hundreds of cycles at deep discharge and high current density to provide the range and performance capabilities which will be demanded.

Advanced secondary batteries will therefore fulfil three roles in the field of efficient energy utilisation, or energy conservation in the medium- to long-term future: (1) load levelling in the electrical generating system; (2) smoothing of supply to demand for alternative energy sources; (3) electric vehicle traction; not only will this reduce dependence on oil supplies but will also improve urban air quality. Additional applications, of large industrial importance, such as nautical or aviation power systems, will, inevitably, also be found.

The second generation of advanced batteries

Rechargeable battery manufacture is still dominated by the lead-acid battery, but because of its low energy-to-weight ratio this system does not offer a very attractive solution to the roles

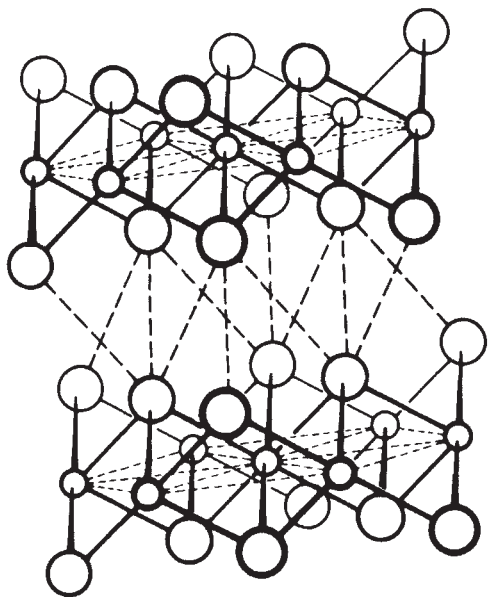


Fig. 4 The cadmium iodide structure adopted by titanium disulphide, TiS_2 . The structure is layer-like with TiS sheets held together only by van der Waals interaction between the sulphur layers. The metal (titanium) atoms are in octahedral coordination by the anions (sulphur).

just outlined. Industrial development of traditional alkaline electrolyte batteries (for example, nickel-zinc and nickel-iron) should produce improved power systems for vehicular traction in the relatively near future; thus the realisable energy density of a nickel-zinc battery is expected to be about 90 Wh kg^{-1} , over twice that of the lead-acid battery.

The present (first) generation of advanced batteries have considerably superior theoretical energy densities to the traditional types, but the weight penalty of engineering safe and reliable sodium-sulphur and lithium-sulphur batteries seems to be considerable, so that the realisable energy density may be no more than 150 Wh kg^{-1} for both these systems and perhaps may be as low as $110\text{--}120 \text{ Wh kg}^{-1}$. The improvement over the anticipated development of the traditional alkaline battery is not now so striking, especially when viewed against the need for high temperature operation, problems of reliability, safety, corrosion and so on. Moreover, it is estimated that the cost per kWh of these high temperature batteries will be similar to those of lead-acid and nickel-zinc; a desirable feature of an advanced battery would be reduced cost as well as improved performance.

Encouraging developments in basic scientific studies on solid-electrolyte synthesis and characterisation, and on solid-solution electrodes, do, however, promise that a new generation of advanced batteries may be possible without the disadvantages observed for sodium-sulphur and similar systems. Improved electrolytes, electrodes and electrochemical understanding of interfacial transport phenomena will, we hope, lead to cheaper, safer, and easier-to-construct second-generation advanced batteries.

The European basis for the new programme

Although much interesting work on new solid electrolytes has been undertaken in several scientific centres in Europe, there is little European industrial involvement so far with second generation ideas. Most of the interesting new developments and patents which might lead towards the second generation of advanced batteries have come from US industry. The example of the Li-TiS_2 battery has already been mentioned.

The Anglo-Danish programme aims to redress this imbalance. Although it will only run in the first instance for 18 months, to the middle of 1979, which is the end of the first 4-yr period of the European Commission's energy conservation programme, indications of success by this time may lead to a larger R & D role for advanced batteries in the next 4-yr programme. Industrial cooperation on promising developments will then be sought.

Although a European perspective for this programme, which is designed to lead to commercially exploitable products, involves a considerable re-orientation of attitude from the national perspective normally taken in such work, it is hoped that the wider view will lead to a better overall assessment of the commercial prospects for advanced battery systems.

The laboratories participating in the programme and the principal investigators, apart from the authors are: UKAEA Harwell Laboratory; Inorganic Chemistry Laboratory, Oxford University (Professor J. B. Goodenough); Department of Metallurgy and Materials Science, Imperial College London (Dr B. C. H. Steele); Department of Ceramics, Leeds University (Professor R. J. Brook); Odense University (Professor J. W. Perram); Danish Technical University, Lyngby (Dr K. Fl. Nielsen); Risø Laboratory (Dr O. Toft Sørensen).

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articles

The Allende meteorite: evidence for a new cosmothermometer based on $\text{Ti}^{3+}/\text{Ti}^{4+}$

Stephen E. Haggerty

Department of Geology, University of Massachusetts, Amherst, Massachusetts 01003

Applications of the mineral thermodynamic condensation sequence to assemblages in Ca–Al–Ti-rich inclusions in the Allende meteorite suggest that the total amount of titanium and Ti^{3+} contents of aluminous clinopyroxenes (fassaite) are temperature sensitive and that the ratio $\text{Ti}^{3+}/\text{Ti}^{4+}$ may be used as a qualitative cosmothermometer.

THE Allende meteorite was an observed fall in Pueblo Allende, Mexico in 1969 and it is classified as a type 3 carbonaceous chondrite. This meteorite has received unprecedented attention because it contains a suite of mineral inclusions which are in agreement with the predicted equilibrium thermodynamic calculations for phases condensing at 10^{-3} atm in the solar nebula¹⁻⁴, and because these inclusions probably represent some of the most primitive high temperature condensates yet encountered⁵⁻⁸. The sequences of mineral condensates, as a function of temperature, are summarised in Table 1 and although there is now uniform agreement that these inclusions, which are rich in calcium, aluminium and titanium, are relics of the primordial nebula, it is still unresolved from the evidence provided by chemical⁹⁻¹⁷, mineralogical¹⁸⁻²⁴, and isotopic data²⁵⁻²⁹, whether condensation ensued directly from a nebular gas to the solid³⁻⁴, whether condensation gave rise to a liquid before solidification¹⁸, or whether the inclusions are the refractory residue of chondritic composition remaining after intense heating and volatilisation^{30,31}.

Formation of inclusions

The differences of opinion on the formation of these inclusions, as primary condensates or as crystallisation products from a melt, depend partly on the interpretation of whether or not equilibrium conditions prevailed during the nucleation of mineral species in the early solar nebula. If the predicted solar thermodynamic mineral condensation sequence is followed (Table 1) we may assume equilibrium, and the calculations are based on an assumption of equilibrium¹⁻⁴. However, if the inclusions are derived by crystallisation from metastable and supercooled condensed liquids, the kinetics of refractory solid–solid reactions argue against a continuation of equilibrium to lower temperatures¹⁸. Provided a distinction is made between inclusions that have high temperature affinities, and inclusions that have lower thermal stabilities, the question of equilibrium is largely reconciled. Only high temperature assemblages are considered here and the thermodynamic model of direct condensation to a solid is favoured.

The refractory inclusions in the Allende meteorite contain a variety of minerals with high titanium concentrations, and evidence is presented here which demonstrates that the valency states of titanium (Ti^{3+} and Ti^{4+}) vary as a function of temperature. Based on the distribution and the abundance of titanium,

and on the systematics of mineral assemblages that are predicted from the thermodynamic calculations, it is proposed that Ti^{4+} and Ti^{3+} may be useful as a potential, but as yet uncalibrated, condensation cosmothermometer.

From the minerals listed in Table 1 and shown in Fig. 1, the major titanium phases in Ca–Al-rich inclusions in the Allende meteorite are: hibonite ($\text{CaAl}_{12}\text{O}_{19}$), 1–10 wt% TiO_2 ; perovskite (CaTiO_3), 58 wt% TiO_2 ; rhonite, $\text{Ca}_4(\text{Ca}, \text{Mg}, \text{Al}, \text{Ti})_{12}(\text{Al}, \text{Si})_{12}\text{O}_{40}$, 12–22 wt% TiO_2 ; and titanian-fassaite (clinopyroxenes in the quaternary system $\text{CaMgSi}_2\text{O}_6$ – $\text{CaAl}_2\text{SiO}_6$ – $\text{CaTi}^{4+}\text{Al}_2\text{O}_6$ – $\text{CaAlTi}^{3+}\text{SiO}_6$), 2–18 wt% TiO_2 . Calculations of

Fig. 1 *a*, The ranges of condensation temperatures for minerals condensing at 10^{-3} atm in the solar nebula; these data are listed in Table 1 and are from Grossman³ and Blander and Fuchs¹⁸. *b*, The same condensation sequence as a function of Ti^{3+} . Titanium-bearing minerals are perovskite, fassaite, Ti_2O_3 , anosovite, TiO_{2-x} , and rutile. Initial condensation temperatures for perovskite and for rutile are labelled limits denoting the upper and lower limits of Ti^{4+} stabilisation; the intermediate range in temperature is the region of Ti^{3+} and Ti^{4+} stabilisation. The dashed curve labelled TiO_{2-x} are Magneli phases or anionic deficient rutiles which may exist in the transitory interval between Ti^{3+} + Ti^{4+} stabilisation and Ti^{4+} stabilisation exclusively. The location of Ti_2O_3 is considered, but dismissed as the peak stabilisation for Ti^{3+} , see text. The dashed and solid curves labelled fassaite are two alternative trends for Ti^{3+} as a function of condensation temperature. The dashed curve is implied because it would provide a symmetrical Ti^{3+} distribution; however, the solid curve is applicable as demonstrated by fassaite-bearing inclusions in Allende with Ti^{3+} decreasing as a function of decreasing T K (see Fig. 2).

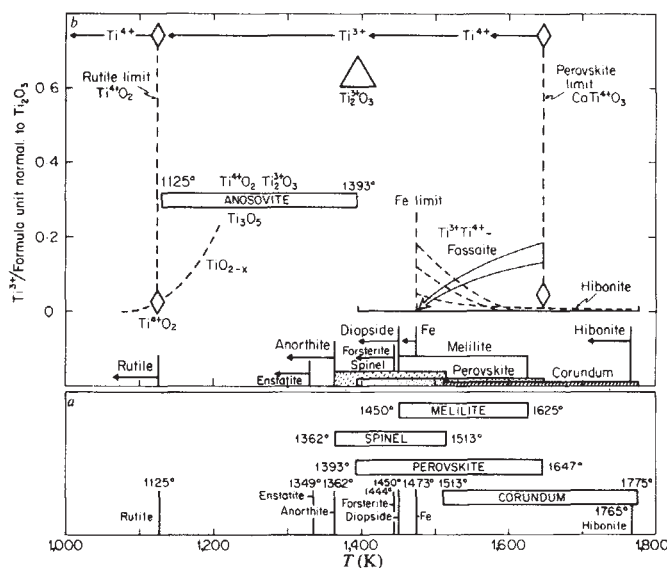


Table 1 The initial and final temperatures of condensation

Phase	Formula	Initial condensation temperature (K)	Temperature of disappearance (K)
Corundum	Al ₂ O ₃	1,775	1,513*
Hibonite*	CaAl ₁₂ O ₁₉	1,765	
Perovskite	CaTiO ₃	1,647	1,393
Melilite	Ca ₂ Al ₂ SiO ₇	1,625	1,450
	Ca ₂ MgSi ₂ O ₇		
Spinel	MgAl ₂ O ₄	1,513	1,362
Metallic iron	Fe(Fe-Ni)	1,473	
Diopside	CaMgSi ₂ O ₆	1,450	
Forsterite	Mg ₂ SiO ₄	1,444	
Anosovite	Ti ₃ O ₅	1,393	1,125
Anorthite	CaAl ₂ Si ₂ O ₈	1,362	
Enstatite	MgSiO ₃	1,349	
Rutile	TiO ₂	1,125	
Albite	NaAlSi ₃ O ₈	~1,000	
Nepheline	NaAlSi ₃ O ₄	~1,000	
Troilite	FeS	405	
Magnetite	Fe ₃ O ₄	≤200	

Values taken from Grossman³, except for * which are from Blander and Fuchs¹⁸.

mineral composition data obtained from electron microprobe analyses, and based on the assumption of mineral stoichiometry, show that >95% of the titanium in perovskite is in the Ti⁴⁺ state, that ~10% of the Ti in hibonite is Ti³⁺, that ~20% of the Ti in rhonite is Ti₂O₃, and that between 24 and 87% of the Ti in titanian-fassaite is also Ti³⁺.

Thermodynamic considerations

Calculations for the predictive thermodynamic condensation sequence show that Ti⁴⁺ is stable in the mineral perovskite (CaTiO₃) at an initial temperature of 1,647 K; that Ti⁴⁺ and Ti³⁺ are stable in anosovite (Ti₃O₅) at an initial temperature of 1,393 K; and that Ti⁴⁺ is once again exclusively stable in rutile (TiO₂) at an initial condensation temperature of 1,125 K (refs 3, 4). This sequence of progressive stabilisation and destabilisation of Ti⁴⁺ is apparently, therefore, inconsistent with the widely held view that the condensing solar nebula becomes increasingly more oxidising with decreasing temperature. This is only an apparent anomaly because metallic titanium is stable at

temperatures above perovskite. The interval between perovskite and rutile is in the interval of Ti³⁺ (and Ti⁴⁺) stability, so that the presence of Ti³⁺ and the ratio of Ti³⁺/Ti⁴⁺ should permit an estimate of the condensation temperature for the titanium-bearing minerals in that intermediate range which is constrained by the temperature limit of perovskite at the high end and by rutile at the lower end (Fig. 1).

To define the temperature range for the initial condensation of titanium-bearing minerals in the Allende meteorite the thermodynamic sequence must be rigorously followed and this sequence must be in agreement with the petrogenesis of the titanium-rich Ca-Al inclusions in Allende. Given these assumptions an analysis of the role of Ti³⁺ and Ti⁴⁺ in the condensation sequence now becomes possible. The absolute upper and lower limits of Ti⁴⁺ stabilisation are defined by perovskite (1,647 K) and by rutile (1,125 K), and values for intermediate Ti³⁺/Ti⁴⁺ values are known for anosovite (Ti₃O₅ or Ti⁴⁺O₂; Ti³⁺O₃) to be in the range 1,393–1,125 K (refs 3, 4). Therefore, the peak stabilisation of Ti³⁺, as Ti₂O₃, ought to be above the upper limit of anosovite (1,393 K). Ti₂O₃ is therefore predictably stable between 1,647 K (perovskite) and 1,393 K (Fig. 1). If this peak stabilisation of Ti³⁺ is correct then the question arises as to whether the transformation of Ti⁴⁺ to Ti³⁺ is progressive on both the high temperature and low temperature sides of Ti₂O₃ (Fig. 1), or whether the transformation is abrupt. With respect to the low temperature side, the synthetic stable occurrences of non-stoichiometric rutile (TiO_{2-x}), known as Magneli phases³²⁻⁴⁶ may be invoked, and the transition from anosovite to rutile may follow a smooth curve of progressively variable Ti³⁺/Ti⁴⁺ concentrations at temperatures above 1,125 K (rutile) but below that of anosovite at 1,393 K (refs 47, 48). This proposed variation is shown in Fig. 1. The decrease towards Ti⁴⁺ stabilisation may also be applicable to the high temperature side of Ti₃O₅; however, an alternative is that the variation is simply a progressive decrease in Ti³⁺, and in total titanium, in the range of condensation from perovskite to anosovite, that a peak in Ti₂O₃ is not attained and that both Ti³⁺ and Ti⁴⁺ are stable between 1,647 K (perovskite) and 1,125 K (rutile).

Discussion and results

To test these hypotheses the electron microprobe analyses of fassaite from the present study and from the literature^{18-24,49} were recalculated assuming mineral stoichiometry, to determine the proportions of Ti³⁺ and Ti⁴⁺ in Allende pyroxenes. These

Fig. 2 Ti³⁺ plotted against total titanium for fassaite and associated pyroxenes and rhonite in inclusions 3898, All-4 and All-3,6. The data are derived from electron microprobe analyses with Ti³⁺ calculated, assuming mineral stoichiometry. ▲, Nos 1-15, and labelled A-I are from the literature listed in Table 2. ●, and the solid, slightly curved lines represent trends in zoned fassaite. Note that the rhonites are plotted on a reduced scale and in reality are at least a factor of two richer in Ti³⁺ than the associated fassaite. The horizontal tie-lines between rhonite and fassaite compositions are for rhonites that have decomposed to fassaite + perovskite + spinel. Open circles represent intra-crystalline fassaite. The dashed line marked ALL-4 is the overall trend for fassaite compositions (130 analyses) in this inclusion; these data are not plotted but are given in Haggerty and Merkel⁴⁷. Arm, armalcolite, mineral 'T' to the new solid solution mineral; R_Σ, products of partial decomposition of mineral 'T' (ref. 53). Fassaite ions are based on 6 oxygens: rhonites are based on 40 oxygens (cation plot = 28/7).

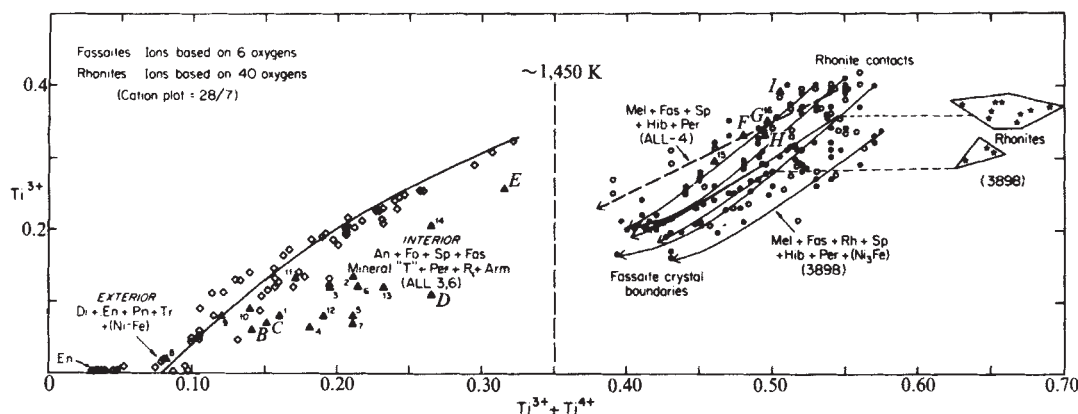


Table 2 Analyses of Ti^{3+} -bearing fassaite

	B	C	D	E	F	G	H	I	1	2	3	4
Mg	0.554	0.554	0.529	0.520	0.382	0.442	0.401	0.289	0.627	0.566	0.567	0.563
Al	0.877	0.806	0.814	0.745	0.871	0.782	0.823	0.989	0.646	0.697	0.719	0.764
Si	1.451	1.481	1.384	1.439	1.258	1.285	1.254	1.196	1.556	1.505	1.509	1.467
Ca	0.977	1.018	1.008	0.979	1.009	0.997	1.027	1.021	1.011	1.000	1.013	1.015
Ti^{3+}	0.060	0.070	0.112	0.254	0.344	0.338	0.324	0.390	0.080	0.128	0.122	0.062
Ti^{4+}	0.081	0.081	0.153	0.062	0.135	0.156	0.171	0.115	0.080	0.084	0.070	0.119
Fe										0.020		0.010
	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Cat. total*	3.97	3.97	3.93	3.92	3.90	3.89	3.89	3.88	3.96	3.96	3.95	3.98
Ti_2O_3	1.92	2.28	3.61	8.17	10.87	10.68	10.21	12.33	2.61	4.14	3.96	2.02
TiO_2	2.87	2.93	5.49	2.22	4.74	5.48	5.99	4.02	2.90	3.02	2.53	4.31
$\text{Ti}^{3+}/\text{Ti}^{3+} + \text{Ti}^{4+}$	0.426	0.464	0.423	0.804	0.718	0.684	0.654	0.772	0.500	0.604	0.635	0.343
	5	6	7	8	9	10	11	12	13	14	15	16
Mg	0.514	0.496	0.453	0.533	0.563	0.563	0.527	0.544	0.484	0.458	0.409	0.381
Al	0.836	0.861	0.947	0.884	0.794	0.774	0.810	0.796	0.869	0.865	0.890	0.907
Si	1.421	1.417	1.350	1.488	1.518	1.518	1.489	1.452	1.394	1.404	1.238	1.216
Ca	0.997	1.003	1.038	1.015	1.005	1.005	1.003	0.997	1.020	1.008	1.003	1.000
Ti^{3+}	0.080	0.122	0.070	0.020	0.080	0.090	0.132	0.080	0.120	0.204	0.288	0.342
Ti^{4+}	0.132	0.091	0.142	0.060	0.040	0.051	0.040	0.111	0.112	0.061	0.172	0.153
Fe	0.020	0.010						0.020				
	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Cat. total*	3.97	3.95	3.97	3.98	3.98	3.98	3.95	3.97	3.96	3.93	3.91	3.88
Ti_2O_3	2.59	3.96	2.26	0.66	2.62	2.94	4.29	2.59	3.88	6.58	9.21	10.80
TiO_2	4.75	3.28	5.10	2.19	1.46	1.85	1.45	4.02	4.02	2.19	6.11	5.37
$\text{Ti}^{3+}/\text{Ti}^{3+} + \text{Ti}^{4+}$	0.377	0.573	0.330	0.250	0.667	0.638	0.767	0.419	0.517	0.770	0.626	0.679

Analyses as listed by Mason⁴⁹, and are from the following sources: B, E, and H from Gray *et al.*⁷; C from Clarke *et al.*⁹; D from Marvin *et al.*⁶; F from Dowty and Clark⁵⁰; G and I from Fuchs²⁰. Analyses listed numerically (1–16) are from Grossman²².

* Cationic totals assuming that Ti total is TiO_2 . Any deviation to values < 4.0 suggests Ti^{3+} . Recalculated TiO_2 , Ti_2O_3 and normalised formulae assume stoichiometry.

results are presented in Tables 2 and 3 and Fig. 2. Although the confidence levels in these calculations are perhaps no greater than 95% it is relevant that independent optical spectra data^{50,52} have demonstrated that Ti^{3+} is present in Allende pyroxenes and that the ratio of $\text{Ti}^{3+}/\text{Ti}^{4+}$ in the fassaite studied is equal to 0.72 (Table 2 analysis F). Particularly close attention was given to monitoring the Ti standard during the microprobe analyses of the present study and repeated analyses were carried out so that the results are not only internally consistent, but are also consistent with data obtained by eight other laboratories. Although the bulk mineralogy of the inclusions from the literature cannot be directly related to those from this study there is uniform agreement among the data that Ti^{3+} is a major component of titanian-fassaite in the Allende meteorite. The proportion of Ti^{3+} correlates positively with total Ti content; and negative and positive correlations exist between Ti^{3+} and Al, and between Ti^{3+} and Si, respectively. These data, although broadly comparable among inclusions, are clearly related to a variety of inclusions which may, or may not, represent precisely identical thermal condensation intervals (Fig. 2).

Fassaite data from three coarse-grained Ca–Al–Ti-rich inclusions (0.5–1.0 mm in diameter) from Allende are shown in Fig. 2 as a function of Ti^{3+} against $\text{Ti}^{3+}/\text{Ti}^{4+}$. These inclusions, designated 3898, All-4 and All-3,6, contain the following assemblages: inclusion 3898 has rhonite as the earliest formed mineral. Rhonite forms cores which are mantled by titanian-fassaite and these rhonites are partially decomposed to spinel + perovskite + titanian-fassaite. The other minerals present are melilite + spinel + hibonite + perovskite. Minute blebs of Fe–Ni alloys occur along cracks in the inclusion in association with grossular garnet, and these minerals are considered to be secondary or reaction condensation products. Inclusion All-4 has the assemblage melilite + titanian-fassaite + spinel + hibonite + perovskite. Inclusion All-3,6 has two contrasting

assemblages: an interior assemblage of anorthite + forsterite + spinel + titanian-fassaite + armalcolite + perovskite + a new mineral series along the solid solution join $\text{R}^{2+}\text{Ti}_3\text{O}_7$ – $\text{R}_2^{3+}\text{Ti}_2\text{O}_7$ (where R^{2+} = Ca, Mg, Mn, Fe; and R^{3+} = Ti^{3+} , Cr, Al); and an exterior assemblage of diopside + enstatite + pentlandite + troilite + Ni–Fe alloys (Fig. 2).

The titanium contents of the fassaite from these three inclusions fall into two populations: one that is high in titanium and a second that is somewhat lower in titanium. The rhonite within fassaite crystals contains the highest total Ti concentration and the highest Ti_2O_3 value (20 wt% Ti_2O_3). The associated fassaite are compositionally zoned, contain 16 wt% Ti_2O_3 at rhonite contacts and a minimum of 6 wt% Ti_2O_3 at fassaite crystal margins. Fassaite in inclusion All-4 have an average Ti_2O_3 content of 9.94 wt% (range, 8.1–14.4 wt%), and an average TiO_2 content of 4.92 wt% TiO_2 (range, 2.04–6.73 wt%), obtained from 125 electron microprobe analyses. These data lie along a relatively smooth curve as summarised in Fig. 2 and are distinguished from the data for inclusion 3898 which occupy a broad band in the distribution of Ti^{3+} and total titanium. Fassaite in the interior of inclusion All-3,6 range from 7 to 10 wt% Ti_2O_3 whereas those on the margins of the inclusion decrease progressively to < 1 wt% Ti_2O_3 .

The condensation temperature for diopside ($\text{CaMgSi}_2\text{O}_6$), a member of the multicomponent pyroxene system, is calculated to be 1,450 K. It is reasonable, therefore, to assume initially that the more complex pyroxenes are within the range of this value, but that higher condensation temperatures are to be expected because of the extraordinarily high Ti and Al concentrations. Several temperature constraints for titanian-fassaite become apparent if the mineralogies of the Allende inclusions are integrated into the thermodynamic condensation temperature model (Table 1). These are as follows: (1) titanian-fassaite is paragenetically earlier than melilite ($\text{Ca}_2\text{Al}_2\text{SiO}_7$ – $\text{Ca}_2\text{MgSi}_2\text{O}_7$),

which has a condensation temperature in the range 1,625–1,450 K, this defines an upper limit of $>1,625$ K; (2) spinel (MgAl_2O_4) overlaps paragenetically with titanian-fassaite so that although the melilite upper limit remains fixed, the spinel (1,513–1,362 K) lower limit of 1,362 K now becomes effective; (3) titanian-fassaites are also present in association with forsterite (initial condensation $T = 1,444$ K) and anorthite (initial condensation $T = 1,362$ K), but not with enstatite (initial condensation $T = 1,349$ K), and this distribution now moves the lower limit from 1,362 K (spinel) to 1,349 K. Condensation temperatures for titanian-fassaites, therefore, are considered to be in the range $>1,625$ K–1,349 K.

Assuming this temperature range, and in association with the mineral chemistries of the three inclusions (3,898, All-4 and All-3,6), relative temperatures of initial condensation may be

The complex but systematic decreases in zoned titanian-fassaites in inclusion 3898 (Fig. 2), the larger bulk titanium contents of fassaite at rhonite decomposition boundaries, and the relative displacements of fassaite titanium concentrations between inclusions characterised by melilite (3898 and All-4), and inclusions dominated by anorthite + forsterite (All-3,6), suggest that titanium dissolution in aluminium-bearing clinopyroxenes is temperature dependent and that these concentrations reflect the environment of solar nebula condensation. These data, therefore, show that total titanium and the proportion of Ti^{3+} decrease progressively with decreasing condensation temperature and that a peak in Ti^{3+} stabilisation, as the oxide Ti_2O_3 , is not attained. The overall trends in Ti^{3+} distributions presented here may only be part of a more complex picture. Inclusions having melilite and anorthite, or inclusions in

Table 3 Electron microprobe analyses for fassaites in inclusions 3898 (1–4), ALL-4 (5–8) and ALL-3,6 (9–12)

	1	2	3	4	5	6	7	8	9	10	11	12
MgO	7.46	6.55	5.62	6.46	6.44	6.54	6.19	6.95	16.55	26.74	22.04	20.67
Al_2O_3	18.37	19.32	20.24	19.01	19.22	19.57	20.24	19.74	7.24	5.33	4.28	3.87
SiO_2	32.82	31.73	29.75	32.40	33.13	33.33	32.94	33.87	45.59	50.74	51.27	49.89
CaO	25.69	25.72	25.68	25.01	25.17	25.15	25.05	25.47	19.49	10.72	18.95	18.41
TiO_2	15.88	16.92	18.89	17.48	16.27	15.39	15.88	14.44	11.07	6.57	3.24	3.61
Cr_2O_3	0.1	0.07	0.07	0.08	0.01	0.02	0.01	0.01	0.53	0.44	0.26	0.32
MnO	0.06	0.08	0.08	0.01	0.05	0.03	0.04	0.02	0.01	0.06	0.08	0.22
FeO	0.01	0.04	0.03	0.01	0.03	0.04	0.04	0.04	0.09	0.31	0.34	2.45
	100.4	100.43	100.36	100.4	100.32	100.07	100.49	100.54	100.56	100.91	100.46	99.44
Mg	0.423	0.373	0.323	0.369	0.366	0.372	0.351	0.392	0.910	1.399	1.169	1.119
Al	0.823	0.870	0.919	0.858	0.866	0.881	0.908	0.880	0.315	0.220	0.18	0.166
Si	1.247	1.212	1.145	1.240	1.266	1.273	1.255	1.281	1.680	1.781	1.823	1.811
Ca	1.047	1.053	1.060	1.026	1.031	1.029	1.027	1.032	0.770	0.403	0.722	0.716
Ti^{3+}	0.229	0.267	0.306	0.348	0.324	0.302	0.316	0.256	0.304	0.140	0.005	0.00
Ti^{4+}	0.224	0.218	0.241	0.155	0.143	0.139	0.138	0.155	0.002	0.032	0.080	0.099
	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Ti_2O_3	7.22	8.38	9.51	10.879	10.24	9.57	10.00	8.13	9.89	4.79	0.20	0.00
TiO_2	7.85	7.60	8.32	5.388	5.02	4.89	4.85	5.47	0.08	1.25	3.02	3.61
Av*	10	8	12	23	28	10	19	15	5	6	4	3

* Number of analyses averaged.

estimated. Because rhonite has re-equilibrated to titanian-fassaite + spinel + perovskite in 3898, and because rhonite has the largest concentration of titanium and the highest proportional content of Ti^{3+} , it is assumed to have condensed at $T > 1,625$ K, but $T < 1,647$ K (initial condensation T K of perovskite). Rhonite may have condensed before perovskite but that cannot be unequivocally demonstrated. Inclusion All-4 is inferred to have condensed in the interval $T = 1,450$ –1,625 K, based on melilite stability; it may have condensed at temperatures slightly higher than 1,625 K but not as high as 1,647 K, or as high as the rhonite-bearing inclusion 3898. The lower limit of condensation for inclusions 3898 and All-4 is defined by the condensation temperature of iron at 1,473 K. Neither of these inclusions have either metallic iron or FeO in mineral analyses and the terminal condensation temperature must, therefore, have been higher than 1,473 K. Inclusion All-3,6 is clearly a lower temperature condensate based on the assemblage anorthite + forsterite + diopside + enstatite (Table 1, Fig. 1), the presence of Ni-Fe alloys, and the presence of FeO in spinel, armalcolite, olivine, the new mineral series ($\text{R}^{2+}\text{Ti}_3^{4+}\text{O}_7$ – $\text{R}_2^{3+}\text{Ti}_2^{4+}\text{O}_7$), and titanian-fassaite⁵³. The upper limit of condensation, therefore, is $T < 1,473$ K, the initial condensation temperature of metallic iron. The lower limit for this inclusion is defined by enstatite, at the margins of the inclusion, at $T < 1,349$ K, but reaction continued in the condensing nebula to temperatures as low as 405 K (troilite) before incorporation into the carbonaceous matrix.

which the evolution of perovskite can be traced, are clearly required to provide information on the behaviour of Ti^{3+} at $\sim 1,450$ K (Fig. 2), and to establish whether the pyroxene trends are duplicated by other titanium bearing minerals.

Conclusions

I conclude that: (1) the calculated thermodynamic condensation mineral sequence is consistent with assemblages and compositions of phases in the high Ca–Al–Ti inclusions in the Allende meteorite; (2) because the predicted thermodynamic sequence is followed, the direct gas to solid condensation hypothesis is favoured; (3) based on these thermodynamic calculations the condensation interval of Ti^{3+} stabilisation is in the temperature range 1,647–1,125 K; (4) the Allende inclusion evidence and thermodynamic constraints suggest that titanian-fassaites condensed in the temperature interval defined by an upper limit of 1,625 K and a lower limit of 1,349 K; (5) the presence of Ti^{3+} in titanian-fassaites may be used as a qualitative cosmo thermometer, with the ratio of $\text{Ti}^{3+}/\text{Ti}^{3+} + \text{Ti}^{4+}$ and total Ti content being high at high condensation temperatures and lower at correspondingly lower condensation temperatures; and (6) rhonite-bearing inclusions have high initial condensation temperatures, which are followed by inclusions with titanian-fassaite and melilite, and these in turn are followed at lower temperatures by inclusions with anorthite + forsterite + diopside + enstatite, suggesting that the Allende meteorite has sampled a spectrum of the condensing solar nebula.

It is emphasised that the ranges of temperatures that are estimated for minerals in the Ca–Al–Ti rich inclusions in the Allende meteorite are subject to the limits of accuracy in the thermodynamic data. However, improvements to these data are unlikely to alter the overall conclusions, although the temperature estimates will undoubtedly be refined.

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Actinide activities in water entering the northern North Sea

C. N. Murray* & H. Kautsky

Deutsches Hydrographisches Institut, Laboratorium Sülldorf

M. Hoppenheit

Biologische Anstalt Helgoland, Laboratorium Sülldorf

M. Domian

Deutsches Hydrographisches Institut, Laboratorium Sülldorf, Hamburg, FRG

Surface seawater measurements of $^{239+240}\text{Pu}$, ^{238}Pu , ^{241}Am and ^{137}Cs have been carried out in the North Sea and western Atlantic. Statistical analysis demonstrated that the discharges by fuel reprocessing plants in the UK are affecting plutonium activities in these sea areas. A difference in behaviour of americium to plutonium was also observed.

THE North Sea is a multi-use coastal-sea area of great economic importance to European countries. As a development of the data collection on actinide element distribution in this region, a detailed investigation was undertaken in July–September 1976 by the FS Meteor of the northern North Sea and Western Atlantic, northwards of 56°30' N till 65°00' N bounded by 10°00' W to about 8°00' E. Fifty-eight sampling stations were measured for surface water activity of $^{239+240}\text{Pu}$ and ^{238}Pu ; ^{241}Am measurements were carried out at 16 stations. Caesium, strontium, ruthenium and tritium measurements were also undertaken and will be reported elsewhere (H.K. in preparation).

The number of results obtained in these sea areas permitted a statistical analysis of $^{239+240}\text{Pu}$ and ^{137}Cs data for variations in

distribution, this was carried out for three different sea regions. An initial estimate has been made of the dispersion of plutonium and the identification of its source as being other than from fallout determined.

Methods

Collection and analysis of actinides and caesium have been described in detail elsewhere^{1,2}. These methods have been used on IAEA intercalibration samples and the results obtained compared with other laboratories by Murray and Stathan¹. Sampling techniques and statistical treatment for these intercalibration samples are discussed in detail by Fukai and Murray³.

The present sampling procedure, which measures the total actinides in unfiltered samples, was carried out on-board the FS Meteor. A series of samples, from two stations, were analysed on-board and in Hamburg to demonstrate that the precipitation carried out at sea and subsequent handling had no systematic effect on the results. No differences in the water activities obtained by the two methods could be detected. Monitoring of the chemical yield solutions before and after the cruise showed that no changes had occurred in their activity during the 2-month period.

The reproducibility of the method for both plutonium and americium was checked by taking replicate sets of samples at three stations, those at 58°36' N, 02°52' W; 58°30' N, 05°29' W

*Present address: Commission of the European Communities, JRC Ispra Establishment, Chemistry Division, 21020 Ispra (Varese), Italy.

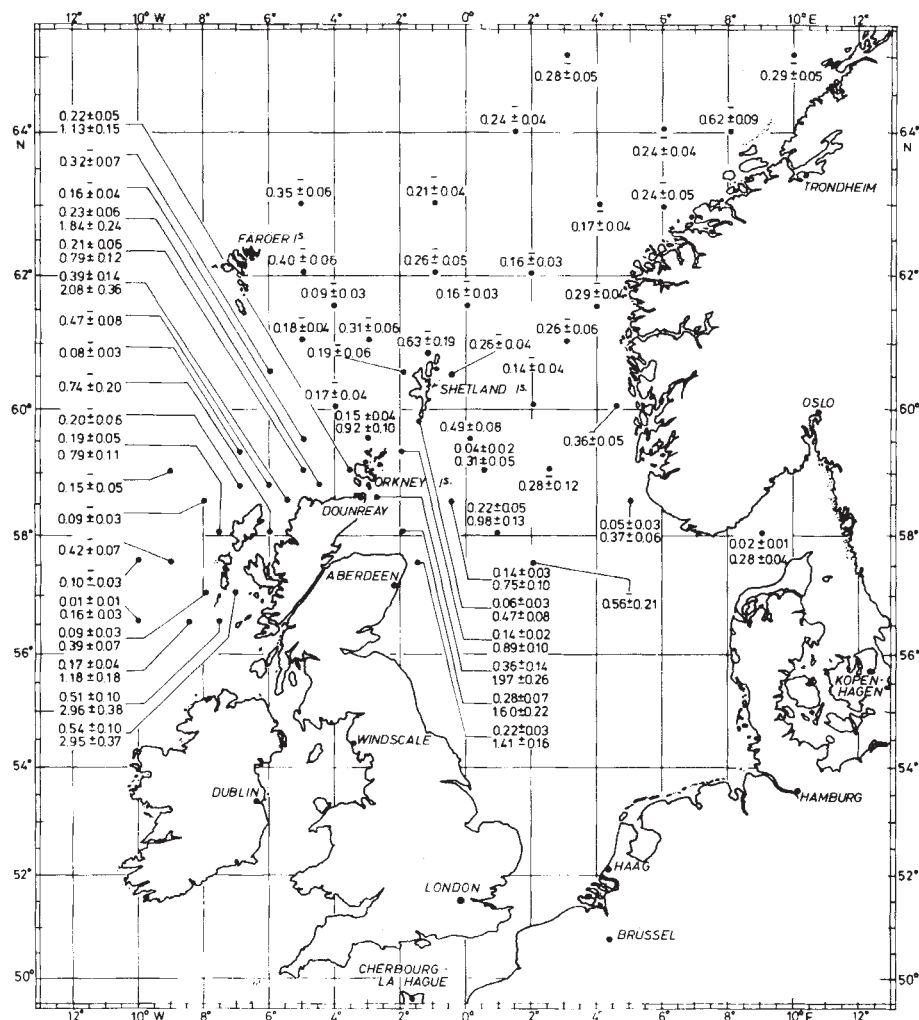


Fig. 1 ^{238}Pu , $^{239+240}\text{Pu}$: activity concentrations in Scottish coastal, North Sea and Atlantic surface waters (fCi kg^{-1}).

and $57^{\circ}00' \text{N}$, $07^{\circ}30' \text{W}$. One sigma (1σ) deviation from the mean values for $^{239+240}\text{Pu}$, ^{238}Pu and ^{241}Am , of three samples for plutonium and two samples for americium for each of three stations were $\pm 14\%$, $\pm 24\%$ and $\pm 28\%$ at activity concentrations of the above isotopes of 2, 0.4 and 0.2 fCi kg^{-1} respectively. Results varied within the single sigma propagated error of the measurements. Reagent blanks were carried out regularly within the chemical separation scheme. All results are given with a 1σ propagated error.

Results and discussion

Figure 1 shows the surface water activity of $^{239+240}\text{Pu}$; values of ^{238}Pu are shown where they were measurable in the volumes analysed. Using the ratio $^{239+240}\text{Pu}/^{137}\text{Cs}$ as a criterion for partitioning the sea area under investigation into regions of comparable ratios, three zones A, B and C were clearly distinguishable; these are shown in Fig. 2. Zone A comprises the Scottish and Norwegian coastal areas and the North Sea and has $^{239+240}\text{Pu}/^{137}\text{Cs}$ ratios of $<0.5 \times 10^{-3}$; zone B, an intermediate area has ratios between 0.5 and 1.0×10^{-3} ; and zone C, the Atlantic region, has ratios $>1.0 \times 10^{-3}$.

It is well documented^{2,4,5} that increased ^{137}Cs activities measurable in the northern North Sea and along the east coast of the UK are due to the operation of the nuclear fuel reprocessing plants at Windscale and Dounreay.

The first increases in ^{137}Cs above fallout levels in water entering the southern North Sea through the English Channel were detected by Kautsky² in 1969, higher water concentrations being measured as far as the Belgian and Netherlands coastlines. The first indication in the northern North Sea was detected by

Kautsky in 1971, along the northeastern coast of Scotland. Successive investigations carried out in 1971 and 1972 indicated that the caesium was being transported southwards along the east coast of England.

In water coming from the English Channel, Kautsky² observed that caesium was transported along the Holland and German Coasts. At this time very little northwards movement towards the southeastern coast of the UK was detected. By 1972 Kautsky² was able to show that some mixing of waters containing ^{137}Cs originating from UK and French fuel reprocessing plants took in the southern North Sea.

Studies by Murray and Eicke⁶ in 1975 on actinide concentrations in sediment and water of the German Bight showed that they were of fallout origin, the $^{238}\text{Pu}/^{239+240}\text{Pu}$ ratio being 0.06 ± 0.02 . Data obtained from the English Channel region for 1975 Murray *et al.*⁷ for ^{241}Pu showed that the apparent detectable limit of actinide intrusion into the southern North Sea, at this time originating from Cherbourg, seemed to lie around 52°N . Apparently, therefore, actinides in the northern North Sea area of the present study must be principally due to sources other than the French reprocessing plant.

Caesium concentrations in the eastern North Sea along the Danish and Norwegian coasts are probably due to mixing of waters containing ^{137}Cs originating from both UK and French reprocessing plants; however, in the central and northwestern North Sea the data of Kautsky^{2,5} show that the ^{137}Cs concentrations depend most probably on the UK plants only.

Recent data by Livingston and Bowen⁸ have shown that plutonium originating from Windscale is measurable in the waters and sediments of the Minch. Data have also been published by Murray and Kautsky⁹ that show that increased levels of

$^{239+240}\text{Pu}$ can also be measured in waters along the north-east coast of the UK and they proposed that these might also be due to the same source. In the present work, using the fact that the source of ^{137}Cs in these areas is known, statistical comparison of the plutonium and caesium data was undertaken to determine whether this source was also responsible for the actinide distribution measured in the North Sea.

Statistical considerations

The whole sea area under consideration has been divided into three major zones A,B,C shown in Fig. 2: the areas have been defined on the basis of the $^{239+240}\text{Pu}/^{137}\text{Cs}$ ratio as has been previously mentioned. Because of the small number of measurements made along the Norwegian coast, the results of this region, which on the basis of its plutonium to caesium ratios belongs to zone A (Scottish coastal area and the North Sea) have not been included in the statistical analysis.

Zone A has been subdivided into two parts, west and north of the Scottish coast, and the North Sea; these are separated in Fig. 2 by a chain dotted line. For both sub-areas the relationship between $^{239+240}\text{Pu}$ and ^{137}Cs is very close. As bivariate normal distributions of the data cannot be expected the rank correlation coefficient r_s (Spearman) has been taken as a measure of the closeness of the relationships. For the west and north areas of the Scottish coast, a rank correlation coefficient (r_s) of 0.95 ($\alpha < 0.001$) has been calculated; for the North Sea area the coefficient is 0.93 ($\alpha < 0.001$). For the intermediate area (zone B) a correlation coefficient for the relationship between $^{239+240}\text{Pu}$ and ^{137}Cs of 0.78 ($\alpha < 0.005$) has been found; this is similar to that determined for stations in the Atlantic (zone C)

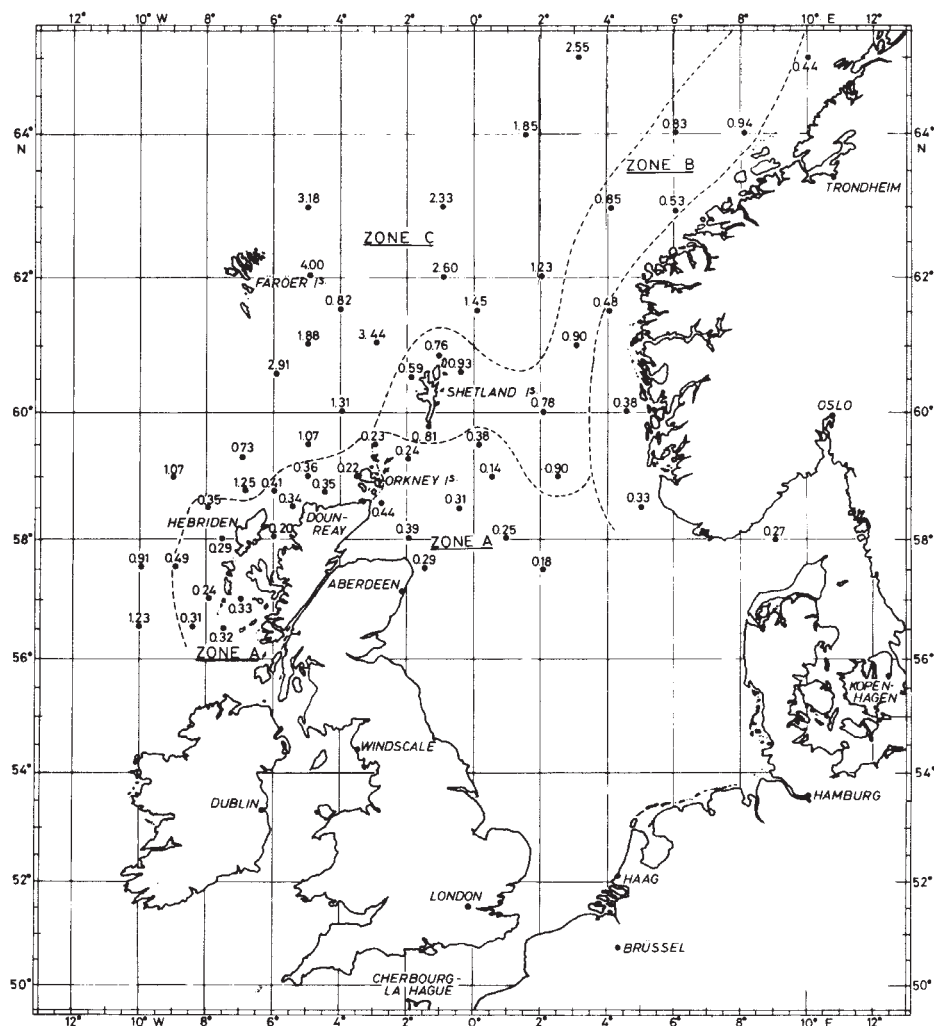
lying south of $60^\circ 15' \text{N}$, of 0.74 ($\alpha < 0.05$). For stations in this zone lying north of this latitude a negative relationship has been estimated of -0.27 ($\alpha > 0.10$). Due to the fact that (except in the case of the North Sea area) there are tied ranks and/or samples of more than 10 pairs, the reliability of the coefficients could be only approximately determined (see ref. 10).

Judging by the nearly equal and high correlation coefficients in the two sub-divisions of zone A, it may be concluded that a major source not only of ^{137}Cs but also of $^{239+240}\text{Pu}$ in these areas is the discharge of low level radioactive waste from nuclear fuel reprocessing plants in the UK; the major activity coming from the Windscale discharge (some thousands of curies) with less than 5 Ci Pu y^{-1} coming from Dounreay. The contribution of the French plant to the ^{137}Cs concentration in these areas (other than the eastern areas of the northern North Sea) is considered to be small in comparison to the UK discharges. Adjacent areas of the Atlantic also seem to be affected by the UK releases.

Further data supporting the fact that the distribution of both isotopes can be accounted for by a similar mechanism of dispersion and source, even though ^{137}Cs is in a soluble cationic form and plutonium supposedly as an insoluble poly-hydroxy-complex, were obtained using the fact that ^{238}Pu activity could also be measured in Scottish coastal and North Sea waters. Figure 3 shows the activity concentration ratios of ^{238}Pu to $^{239+240}\text{Pu}$. Visual examination of the data showed clearly that there was a strong correlation between the isotope concentrations; the rank correlation coefficient was found to be 0.96 ($\alpha < 0.001$) and thus confirmed the relationship.

To estimate the ratio $^{238}\text{Pu}/^{239+240}\text{Pu}$ a regression line of the data (Fig. 4) from the regions was calculated by the method of least squares. The resulting equation $y = 0.1756x - 0.0007$

Fig. 2 Activity concentration ratios of $^{239+240}\text{Pu}/^{137}\text{Cs}$ multiplied 10^3 in Scottish coastal, North Sea and Atlantic surface waters.



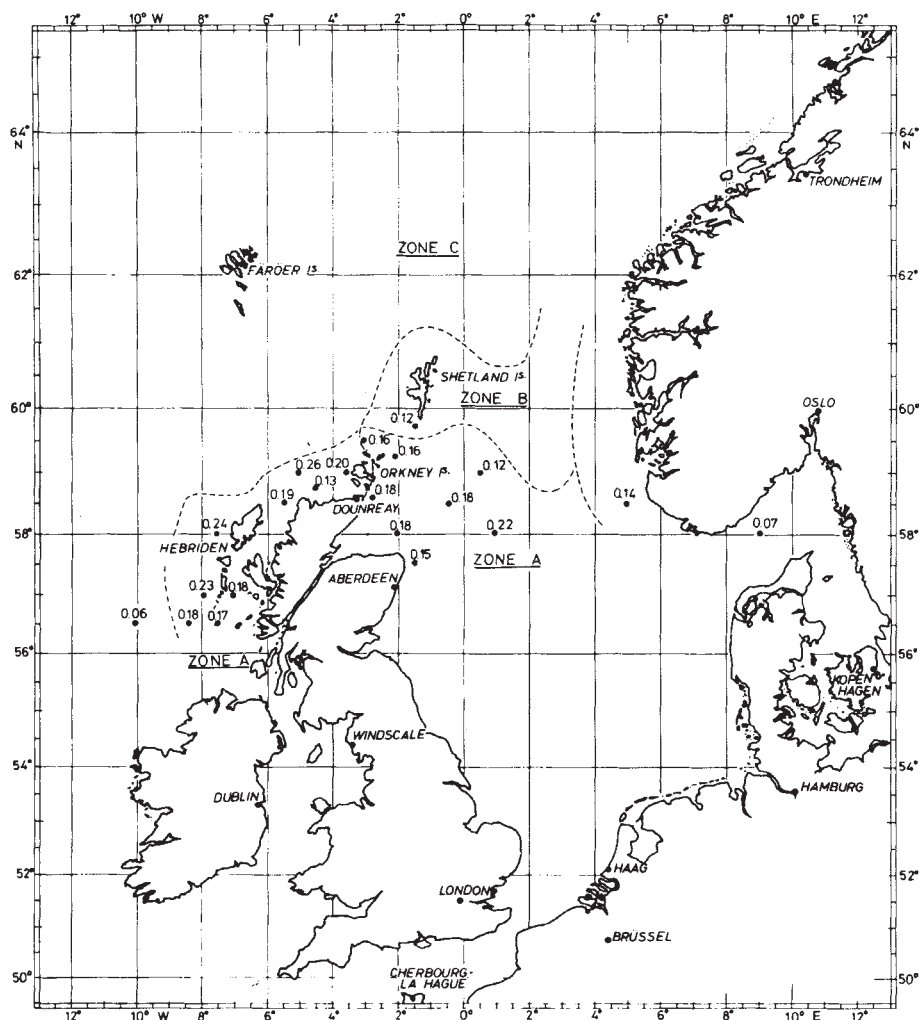
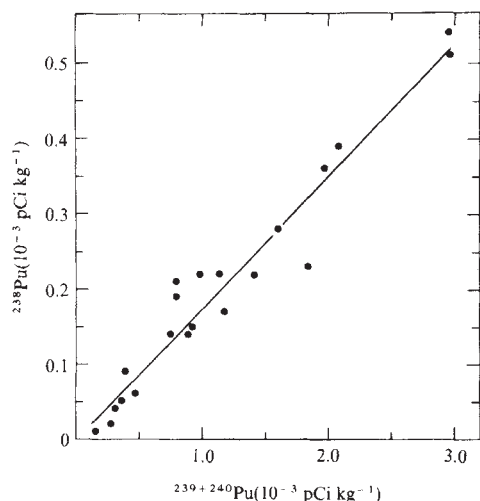


Fig. 3 Activity concentration ratios of $^{238}\text{Pu}/^{239+240}\text{Pu}$ in Scottish coastal, North Sea and Atlantic surface waters.

indicates that the regression line, as expected, goes through the origin. As both variables are subject to error the regression coefficient is distorted downwards. Estimated propagated errors have been used to calculate the correction factor for the regression coefficient according to the procedure proposed by Snedecor and Cochran¹¹; the corrected coefficient was found to be 0.1797. As can be recognised, the downward bias is small. The 99% confidence limits of the regression coefficient have been

Fig. 4 ^{238}Pu , $^{239+240}\text{Pu}$ activity concentrations. Regression line: $y = 0.1756x - 0.0007$; $r_s = 0.96$; $\alpha < 0.001$.



estimated to be 0.1465 and 0.2129; the estimated ratio therefore of $^{238}\text{Pu}/^{239+240}\text{Pu}$ in Scottish coastal and North Sea waters is 0.18 with 99% confidence limits of 0.15 and 0.21 and is significantly different from the ratio of 0.066 ± 0.008 (1σ limits propagated error, figures calculated from data of Fukai *et al.*¹²) found for samples contaminated only from fallout plutonium in the Northern Hemisphere. Hardy *et al.*¹³ report ratio results $^{238}\text{Pu}/^{239+240}\text{Pu}$ of about 0.04 for integrated samples (undisturbed soils) from the Northern Hemisphere 1970–71; the standard deviation of the average ratio being $\pm 12\%$. At the 2σ level the ratios given in refs 12, 13 are indistinguishable and are significantly different from the ratios found in this present study.

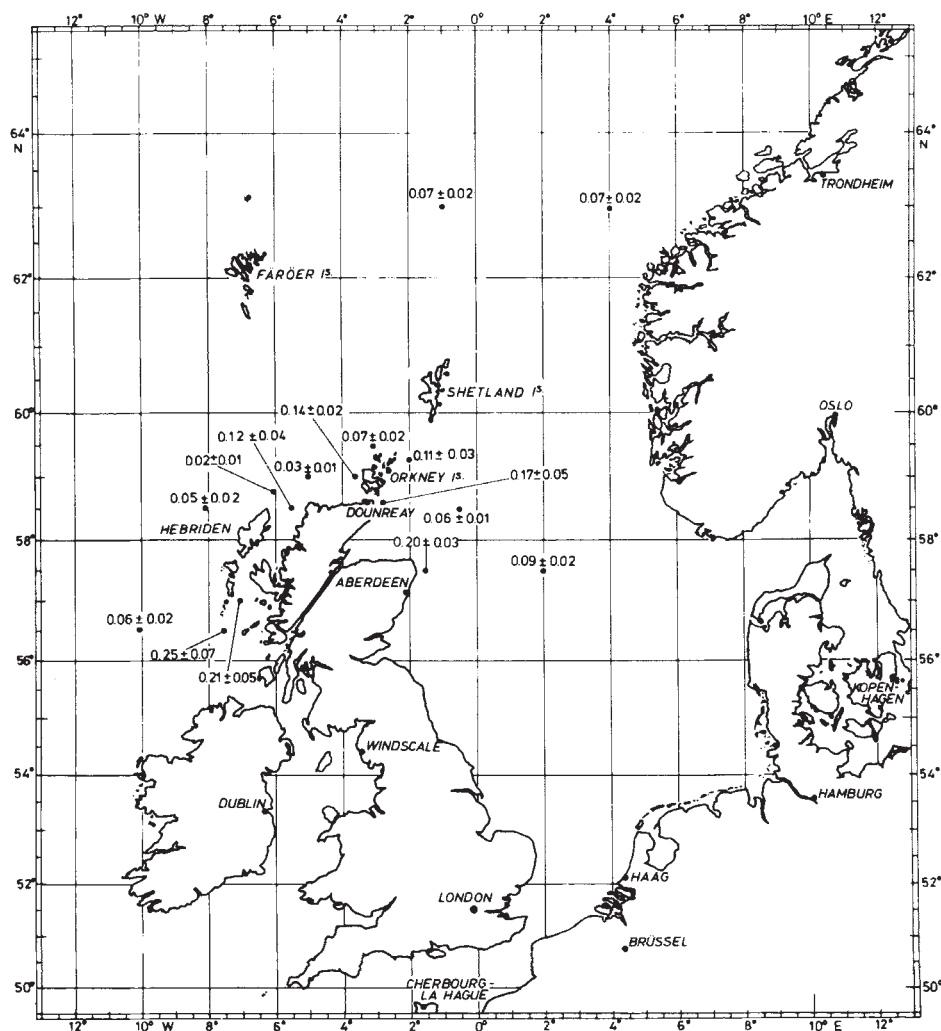
It may be concluded that, as with ^{137}Cs , quantities of plutonium originating from the discharge of waste from nuclear fuel reprocessing plants into UK coastal waters are present in surface waters of the central North Sea and thus have been transported over large distances from their source of origin. An estimate of 1.1–1.8 yr for the transit time of ^{137}Cs from the vicinity of Windscale to the North Channel of the Irish Sea has been made by Jefferies *et al.*⁴ for 1970–72. Two years has been estimated for the Minch by Livingston and Bowen⁸. The time required for its transport along the west and north Scottish coasts and into the North Sea is difficult to estimate, but if one takes an interval of a further 1.5–2.0 yr to pass through the Pentland Firth into the North Sea, this would mean that activities now being measured in the North Sea probably originated from discharges taking place during 1972–73 in the Irish Sea. This seems to coincide with an increased discharge by Windscale of α -containing low level waste that occurred at this time¹⁴. Thereafter discharges apparently decreased, according to data from Hetherington *et al.*^{15,16}.

Figure 5 shows the measured activity levels of ^{241}Am in surface waters. It can immediately be seen that activities near the northwestern coast of Scotland are considerably smaller than those of plutonium; the ratio $^{241}\text{Am}/^{238,239+240}\text{Pu}$ for three stations between Barra to the Pentland Firth is only 0.06 ± 0.02 . If we assume that the present results for activities in western Scottish coastal waters some 300–500 km north of Windscale are representative of discharges into the Irish Sea that occurred some 2.0–2.5 yr earlier, then this water was most probably contaminated some time in 1973–74. That these present ratios should be so small is surprising in view of the report by Hetherington *et al.*¹⁷ who gave the Am/Pu α -radioactivity discharge from Windscale for 1971–74 as varying between 0.9 and 2.56. It seems that there has been a decrease in the ratio from about 1.7 at Windscale (average value 1971–74) to 0.06 some 300–500 km northwards along the Scottish coast. This

still more than 50%. The settling out of suspended particles from surface water thus tends to decrease the americium activity more quickly than plutonium.

In the present survey no measurable quantities of ^{242}Cm were detected in samples near the Scottish coast even though the chemical separation scheme we used is capable of detecting it at the same levels as for americium. Although the lack of ^{242}Cm activity in water could be due to the low concentrations discharged into the Irish Sea, Hetherington *et al.*¹⁶ report it at levels similar to ^{238}Pu in the seaweed porphyra collected from the Cumberland coast. The fact that ^{242}Cm was not detected in Scottish coastal samples although apparently present in water off the Cumbrian coast probably reflects the short half-life ($t_{1/2} = 163$ d) of this isotope compared to the time taken for the water to travel that far north. The non-detection of curium is, however, also consistent with data obtained from the English

Fig. 5 ^{241}Am activity concentrations in Scottish coastal, North Sea and Atlantic surface waters (fCi kg^{-1}).



decrease takes place in spite of the increase of ^{241}Am that will have occurred during its transport due to the decay of ^{241}Pu .

Our hypothesis that americium disappears more quickly from surface waters than plutonium is supported by data of Hetherington and Harvey¹⁸ on the distribution of these elements between suspended material and solution in Irish Sea surface water. These workers reported that at a distance <10 km from the point of discharge, the percentage fraction of activity associated with suspended material for plutonium and americium was 36 ± 8 and 80 ± 7 , respectively. The total activity in the water was found to be 2.4 ± 0.8 and 1.7 ± 0.8 pCi l^{-1} for the two isotopes. Thus at a distance of only 10 km from Windscale twice as much americium is associated with suspended material as plutonium; even at a distance of about 100 km this fraction is

Channel (C.N.M., unpublished data), which indicates that curium activity seems to be also lost more quickly from the water column than plutonium.

Conclusions

First, based on a statistical analysis of the distribution of ^{137}Cs and plutonium from samples obtained in July–September 1976, it has been shown that a significant fraction of surface water activity of plutonium in UK eastern coastal waters and the central North Sea originates from fuel reprocessing plants in the UK, the major contributor being Windscale.

Second, the fact that plutonium moves over such relatively great distances, although representing only a small fraction of

that which is initially absorbed to bottom sediments, may have important consequences for considerations of its medium and long-term behaviour in the marine environment, especially in relation to the possible future siting of fuel reprocessing plants in North Sea coastal areas.

Finally, the apparent variations in the distribution of actinide isotopes in seawater, as shown in the present survey, underline the need for care in drawing conclusions about their environmental behaviour on the basis of a simple comparison with plutonium. The possibility of differences in behaviour for actinides coming from different sources must always be considered, when predicting their impact on the environment.

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Surface antibodies of human myelogenous leukaemia leukocytes reactive with specific type-C viral reverse transcriptases

Paul C. Jacquemin, Carl Saxinger & Robert C. Gallo

National Cancer Institute, National Institutes of Health, Laboratory of Tumor Cell Biology, Bethesda, Maryland 20014

Purified immunoglobulin G (IgG) from patients with chronic myelogenous leukaemia specifically neutralised RT from feline leukaemia virus while purified IgG from other types of leukaemias and from normal blood cells were less reactive and in some cases preferentially reacted with RT from horizontally transmitted primate type-C viruses (simian sarcoma virus–gibbon ape leukaemia virus group). This indicates the presence of a heterogeneous immune response to RT or to an RT-like molecule in humans.

SOME human leukaemic cells contain a cytoplasmic DNA polymerase similar to reverse transcriptase (RT) of RNA tumour viruses^{1,2}. In some cases the enzyme has been partially purified^{1,3,4}. Molecular weight, column elution, response to various template–primers and immunological tests distinguish RT from cellular DNA polymerases (α , β and γ)^{1,3–8} and show its similarity to viral RT^{1,3,4}. The activities of some partially purified, RT-like DNA polymerases from patients with acute myelogenous leukaemia (AML) have been specifically neutralised by IgG from hyperimmune sera prepared against RT from the simian sarcoma virus (SSV)–gibbon ape leukaemia virus (GaLV) group^{3,9–12}. RT-like DNA polymerase neutralised by antisera against RT from SSV was also isolated from the spleen of a child with myelofibrosis¹³ (a preleukaemic disease), who later developed AML. These results suggest that the enzymes from some AML cells and some preleukaemic cells are immunologically related to RT from these viruses. Other RT-like DNA polymerases detected in human leukaemic cells were not detectably related to RT from any of the viruses studied, that is, SSV, GaLV, murine leukaemia virus (MuLV) and avian myeloblastosis virus (AMV) (refs 11, 12).

Some reports suggest expression of type-C virus-related structural proteins in human tissues (reviewed in ref. 12) and of antibodies reactive with them^{14–16}, but there are no reports of such interactions with purified proteins. It would be interesting to know whether antibodies to specific RTs can be identified in

humans not only because of the RT-related polymerases but also because RT can be identified unambiguously by its function. We have not found anti-RT activity in human sera (unpublished results) in contrast to published results with two cases of AML¹⁷.

We report here that the surface of some fresh human blood cells contains IgGs specifically reactive with certain viral RTs. The rationale for our experiments was (1) evidence for both the presence of a RT-like DNA polymerase in some leukaemic cells and the absence of detectable antibody to RT in human sera; (2) the precedent in solid tumours for tumour bound antibody¹⁸ which can be eluted and isolated¹⁹; (3) indications of antibody on the surface of human leukaemic cells, the specific reactivity of which had not been elucidated^{20,21}; and (4) indications that antibodies to RT may be present in some naturally infected cats²², gibbons²³ and cows²⁴.

Detection and isolation of surface bound immunoglobulins

Leukocytes were obtained from whole blood from leukaemic or normal people by leukaphoresis or from the buffy coat of 500 ml of anticoagulated blood (acid citrate dextrose). The cells were maintained in a fresh, viable state at 4 °C during transport and washed three times at 4 °C with 10 vol. cold RPMI 1640 medium on receipt in our laboratory. They were assayed for surface Ig by measuring the binding of ¹²⁵I-labelled (ref. 25) F(ab')₂ fragments^{26,27} of goat anti-human (GAH) IgG. In saturating conditions this amounted to 0.2–10 μ g per 10⁶ leukocytes from leukaemic donors and 0.05–0.2 μ g per 10⁶ leukocytes from normal donors.

The cell surface Ig could be eluted into serum-free media. The complexes formed between surface Ig and the ¹²⁵I-labelled GAH–IgG remained cell-bound during incubation at 4 °C overnight but were released by incubation at 37 °C in serum-free medium for 12–16 h as described by Witz¹⁹. The Ig detected on the surface of leukocytes was eluted from washed cells as described in the legend to Fig. 1. IgG was determined by a solid phase immunofluorescent assay (Biorad). The amounts of IgG recovered in the medium were similar to that detected on the intact cell surface by the binding assay. These findings are similar

to results reported after acid treatment of leukaemic blood cells^{20,21}.

The Ig contained in the eluates from AML, chronic myelogenous leukaemia (CML), or normal blood leukocytes were purified as described in the legend to Fig. 1 and analysed at each step of purification by SDS polyacrylamide gel electrophoresis (PAGE). The stained gels from the crude eluates showed 5 to 10 bands, including two constituents with mobilities identical to control standard human IgG heavy and light chains. After immunoaffinity column chromatography the purified IgG on SDS PAGE revealed only two bands corresponding to heavy and light chains of IgG (not shown). The activity of the purified IgG fractions was stable at 56 °C for 1 h, non-dialysable, and not bound to DEAE-Agarose in 20 mM Na phosphate buffer, pH 8.

Inhibition of RT by IgG from CML leukocytes

The purified IgG was tested for inhibition of cellular DNA polymerases α , β , γ and terminal deoxyribonucleotidyl transferase (TdT), purified as before^{5-8,28} from normal or leukaemic human leukocytes and for inhibition of RT purified^{29,30} from various oncornaviruses. We used purified IgG because the

magnitude and specificity of inhibition was greater than with the crude eluates, which contained competing proteins and protease artefacts. Cell surface IgG purified from the blood leukocytes of patients with the acute blast crisis (BC) phase of CML completely inhibited RT purified from some mammalian type-C viruses, most notably feline leukaemia virus (FeLV) (Fig. 1, Tables 1 and 2). Low concentrations of IgG were effective, for example, 50% neutralisation of FeLV RT was obtained with <0.2 μ g using IgG from patient HL68 (Fig. 1a-c, Table 1). Table 1 shows that activity of the human cell surface anti-RT IgG compares quite favourably in specific activity with anti-RT IgG obtained from serum from animals immunised with RT or serum obtained from naturally infected animals. Furthermore, the inhibition was specific. There was no inhibition of cellular DNA polymerases α , β , γ or of TdT (Fig. 1a), nor of RT purified from avian type-C viruses or of RT from non-type-C retroviruses (Fig. 1b). Inhibition was found only with RT purified from some mammalian type-C viruses (Fig. 1c), especially FeLV (Fig. 1a-c). Blood leukocytes from eight of nine patients with CML-BC yielded antibodies with activity specifically against FeLV (Table 2). There was variation in the amount of cross-reactivity with RT from viruses related to FeLV. IgG from some patients showed remarkable type

Fig. 1 Specific neutralisation of viral RT by IgG purified from blood leukocytes of patients with CML, blast crisis. IgG was purified as follows: 10 g of washed blood leukocytes, obtained by leukaphoresis from patient (HL-68) with CML (BC), were incubated for 12 h at 37 °C in serum-free RPMI 1640 medium (10^7 cell ml^{-1}) containing gentamycin ($2.5 \mu\text{g ml}^{-1}$) and fungizone ($2.5 \mu\text{g ml}^{-1}$) to elute membrane-bound IgG. Some proteins in the eluate, including IgG, were precipitated by adding $(\text{NH}_4)_2\text{SO}_4$ to a final concentration of 50%. The precipitate was dissolved in 20 mM Na phosphate, pH 8.0, then dialysed overnight against the same buffer. An aliquot of the dialysed sample was analysed by SDS PAGE. The IgG in the remaining sample, containing 4.5 mg of protein, was further purified on a DEAE Agarose column (10 mg protein per ml gel). The flow-through and 2 one-column vol. washes of 20 mM Na phosphate, pH 8.0, were combined (total protein 1.7 mg) and further purified on an affinity column of Sepharose 4B specific for human IgG. The column was prepared as follows. Purified GAH IgG was coupled to cyanogen bromide activated Sepharose (Pharmacia) in 0.1 M Na phosphate, pH 8.0, at a ratio of 10 mg IgG per ml 'swollen' gel. The reactive groups were blocked with 0.5 M ethanolamine, pH 9.0, washed with 0.85% NaCl and equilibrated in solution A (0.05 M Tris-HCl, pH 7.4, 0.3 M NaCl containing 2% Triton X-100, 0.1% Tween 80, and BSA 10 mg ml^{-1}). One-third of the total protein of the commercial GAH IgG was specific for human IgG. Protein (1.7 mg), containing the IgG antibodies, obtained from the DEAE Agarose step was dissolved in 1 ml of solution A containing the affinity beads and incubated overnight at 0 °C. The slurry was then poured into a column, extensively washed with 0.85% NaCl until the A_{280} was <0.01. Bound IgG was eluted with 0.2 M glycine, pH 2.8 and one ml fractions were collected at 4 °C. Different DNA polymerases were tested against IgG purified on DEAE Agarose and affinity chromatography. The 100% activity corresponds to activity of the different polymerases in the absence of antibody. a-c, Purified IgG from patient HL-63; d, patient HL-105; e, f, patient HL-70. The IgG from patient HL-70 (e, f) were from two different preparations of purified IgG. f shows that RT purified from different strains of FeLV and/or by different methods are comparably neutralised by the IgG. RT was purified from FeLV Rickard (1) by DEAE cellulose, phosphocellulose, poly G Agarose, and hydroxyapatite sequential column chromatography³⁰. Purification of RT from FeLV Rickard (2) and for FeLV Theilen (3) was as previously described²⁹. DNA polymerase (refs 5-7, respectively) and TdT²⁸ were purified from human leukocytes as described. FeLV (Rickard strain) was grown in feline lymphoid cells, F422; FeLV (Theilen strain) was grown in FL74 feline lymphoid cells; and various FeLV subgroups were also grown in NC37 human lymphoblasts and in canine thymus fibroblasts (FCF₂Th). AMV was obtained from chicken plasma. Other viruses are: BoLV (bovine leukaemia virus) grown in sheep fibroblasts; MPMV (Mason-Pfizer monkey virus) grown in NC37 human lymphoblasts; RaEV (Rat endogenous virus) grown in Wistar rat cells; MuLV (AKR) grown in 3T3 mouse cells; GaLV (San Francisco strain), grown in NC37 cells; BaEV, grown in canine thymus cells (FCF₂Th) and in NC37 human lymphoblasts; and HL23V, a virus isolated from a woman with AML which predominantly contains a virus highly related to SSV and less of a second virus highly related to BaEV, grown in FCF₂Th cells. RT were purified from different viruses after disruption in a 0.6 M KCl, 0.5% Triton X-100 by successive chromatography on DEAE cellulose, phosphocellulose, poly G Agarose and hydroxyapatite. In some cases an additional glycerol gradient step completed purification. Details of these and alternative methods are described elsewhere^{29,30}. Assays were in 0.05 M Tris-HCl buffer, pH 7.4 containing 140 μM ^3H -dTTP (3.6 Ci mmol^{-1}); 130 μM dATP; 50 $\mu\text{g per ml}$ dT₁₂₋₁₈ · poly A; 0.5 mM MnCl_2 ; 5.0 mM DTT; and 130 mM NaCl for all viruses with the following exceptions: for AMV RT, 6 mM MgCl_2 was substituted for MnCl_2 ; for BoLV and MPMV RT the conditions were 50 μM ^3H -dTTP (10 Ci mmol^{-1}), 190 μM dATP and 40 $\mu\text{g per ml}$ dT₁₂₋₁₈ · poly A. Activities were measured at 30 °C for 30 min except for AMV, BoLV and MPMV, which were assayed at 37 °C for 30 min. The enzyme and antibody were preincubated at 4 °C for 4 h in the preincubation buffer (0.05 M Tris-HCl, pH 7.4 with 130 or 190 mM NaCl, 2% Triton X-100, 10 mg per ml BSA, and 0.1% Tween 80) and then assayed as described above. The 100% activity was obtained in the absence of antibody and varied between 40,000 and 70,000 c.p.m. (20-40 pmol) of ^3H -dTTP incorporated into acid-insoluble precipitates. Comparable amounts of protein and enzyme units were used for each polymerase.

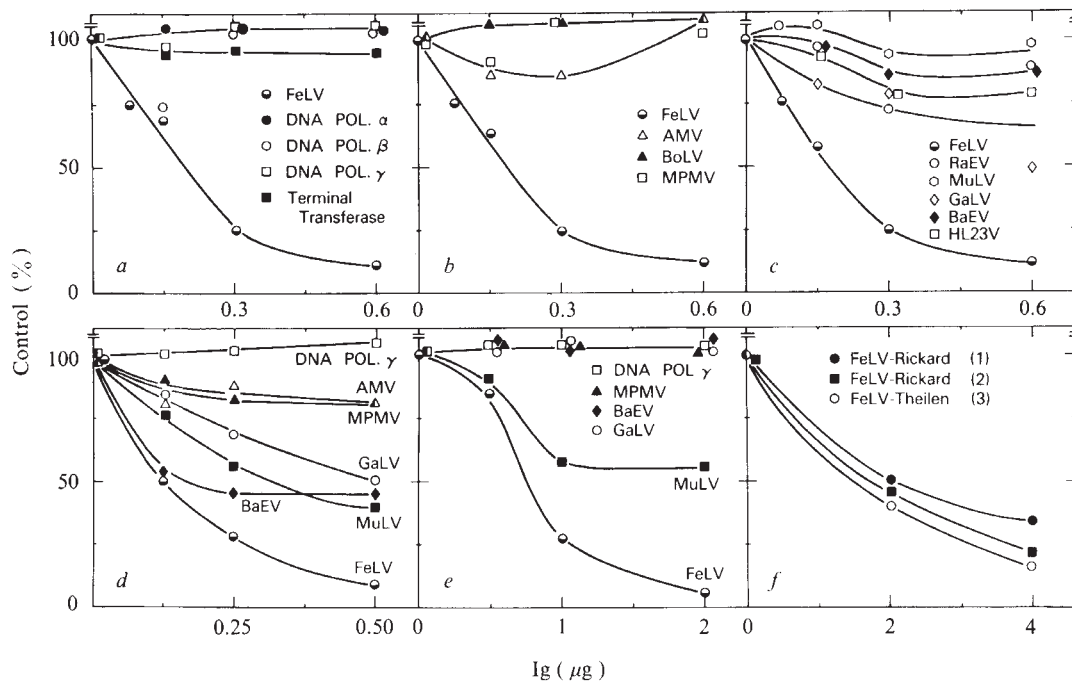


Table 1 Comparison of neutralisation of cellular and viral DNA polymerases by different antibody (IgG) preparations

Source of DNA polymerase*	Source of purified IgG with anti-RT activity†						FeLV-infected cat serum
	Eluates from human blood leukocytes‡				Hyperimmune sera§		
	CML(BC) (HL-68)	CML(BC) (HL-105)	AML(HL-80)	Normal	Anti-MuLV	Anti-GaLV	
		(µg of IgG for 50% inhibition of polymerase)					
Human DNA pol α, β, γ and TDT	>0.6	>0.5	>10	—	—	—	—
Viral							
AMV	>0.6	>0.5	>10	—	—	—	—
MPMV	>0.6	>0.5	—	—	—	—	—
GaLV	>0.6	0.4	>10	0.95	>45	21	—
SSV	—	—	5	>5	27	42	>60
HL23V	>0.6	—	4	>5	22	>60	—
MuLV	>0.6	0.3	>10	>5	9	>60	—
BaEV	>0.6	0.25	>10	>5	—	—	>60
FeLV	0.15	0.125	>10	>5	—	—	15

* Cellular DNA polymerase (pol) γ was a 9,000-fold purified enzyme from human lymphoblasts. Similar results were obtained with DNA polymerases α, β, γ and TDT. Viral polymerases (RT) were purified as described elsewhere^{29,30}. Assay conditions are described in legend to Fig. 1.

† See text and legend to Fig. 1 for IgG purification procedure.

‡ Natural antibodies eluted from human CML blast crisis, AML, and normal blood leukocytes.

§ IgG purified from hyperimmune rabbit sera obtained after inoculation of animals with purified RT from Rauscher MuLV or GaLV.

|| IgG containing specific anti-FeLV RT activity was purified from serum derived from a cat naturally infected with FeLV.

specificity for FeLV with little or no reactivity against RT from other viruses, for example, IgG from patient HL-68 (Fig. 1c), while IgG purified from leukocytes of other patients with CML (BC) included cross-reactivity with RT from rodent type-C viruses (patient HL-70, Fig. 1e) or more extensive cross-reactivity (patient HL-105, Fig. 1d). RT purified by different methods and from different strains (Rickard and Theilen) of FeLV were comparably neutralised by IgG from patient HL-70 (Fig. 1f). Therefore, the specificity in polymerase neutralisation is probably not affected by variation in specific enzyme activity among different enzyme preparations. Also, RT from FeLV grown in several different types of cells (see legend to Fig. 1) was comparably neutralised by this IgG which indicates that cellular components do not influence the specificity of the interaction.

Three separate serial blood samples were studied from patients HL-62, HL-68 and HL-101 with relatively constant

clinical and haematological features. The amount of IgG required to neutralise RT and the specificity pattern for anti-RT activity were constant. Six separate IgG purifications from one blood sample (patient HL-105) and multiple purifications from others also yielded reproducible results (data not shown).

Leukocytes from CML patients in remission (two cases) were negative or contained low levels of IgG not reactive with RT. Of four patients tested with CML in the chronic phase of the disease two were negative and two contained IgG with some inhibition of RT from the GaLV-SSV group (Table 2), especially the San Francisco strain of GaLV (GaLV_{SF}). The IgG most reactive with FeLV RT have been predominantly from a particular type and phase of leukaemia, namely Philadelphia chromosome-positive CML-BC lacking TDT. TDT, an enzyme generally found only in certain types of lymphoblasts³¹⁻³³ was discovered in CML-BC cells²⁸ and subsequently found in about one-third of these patients³³, indicating that these patients may develop an acute lymphoblastic proliferation in contrast to the usual myeloblastic proliferation. The IgG with activity against FeLV RT described here appears then to be associated mainly with CML-BC with acute myeloblastic proliferation.

Table 2 Summary of human blood leukocyte surface IgG with anti-RT activity

Diagnosis and disease stage	Number studied	Number positive	Anti-RT activity
			Specificity of inhibition†
Leukaemic samples			
CML, blast crisis*	9	8	FeLV
CML, chronic phase*	4	2	GaLV _{SF}
CML, remission*	2	0	—
AML, untreated	5	2	SSV (and HL23V)
AMML, untreated	4	1	SSV (and HL23V)
AUL, untreated	2	0	—
Uncertain diagnosis (AML or CML crisis)	1	1	SSV (and HL23V)
ALL	3	0	—
CLL	3	0	—
Normal samples			
PHA stimulated blood lymphocytes (pooled 12 donors)	1	0	—
Bone marrow	4	1	GaLV _{SF}
Buffy coat blood leukocytes	24	7	GaLV _{SF}

* All CML patients were Philadelphia chromosome positive. Cells from those patients in blast crisis were negative for TDT assayed as described elsewhere^{28,33}.

† Positive cases were those in which the RT neutralisation titration showed ≥30% inhibition and no inhibition of control enzymes (control enzymes: see Fig. 1a, b).

IgG from other leukaemic and from normal leukocytes

IgG were also purified from the surface of blood leukocytes from other types of leukaemia. Antibodies reactive with RT were obtained from two of five patients with AML and one of four acute myelomonocytic leukaemia (AMML) patients (Table 2). In general, the IgGs purified from the positive AML and AMML patients mainly inhibited RT from SSV or the SSV-related virus, HL23V³⁴. This was shown with intact IgG (not shown) as well as with a F(ab')₂ fragment derived from the purified IgG (Fig. 2a). The amount of IgG required to inhibit the preferred RT by 50% was much higher (5 µg) than from patients with CML-BC. There was little or no inhibition of cellular DNA polymerases or of RT from AMV, baboon endogenous virus (BaEV), GaLV, MuLV or FeLV (Fig. 2a, Tables 1 and 2). Similar specificity patterns were also obtained with IgG isolated from a blood sample of patient (HL-67) with an uncertain diagnosis (AML or CML-BC). The inhibition of RT by this IgG was specific for SSV and HL23V polymerases (Table 2). We also examined two cases of acute undifferentiated leukaemia (AUL), three cases of acute lymphoblastic leukaemia (ALL), and three cases of chronic lymphocytic leukaemia (CLL) for IgG with anti-RT activity—all were negative (Table 2).

There is no natural source of tissue that provides comparable control cells for human leukaemic blast cells. By using techniques described for leukaemic blood cells, we prepared eluates from various established tissue culture cell lines, for example, bat lung fibroblasts and canine thymus cells and from diverse normal human haematopoietic cells, including peripheral blood buffy coat (24 donors), bone marrow samples (4 donors) and nylon column purified PHA stimulated blood lymphocytes (pooled from 12 donors) from normal individuals. None of the tissue culture cells or the cultured human blood lymphocyte eluates contained RT inhibitory activity, but 8 of 29 fresh normal human haematopoietic cell samples exhibited significant inhibitory activity for RT from mammalian type-C viruses, particularly RT from GaLV_{SF} (>40% inhibition with 5 μ g of IgG). All positive results were obtained with bone marrow or

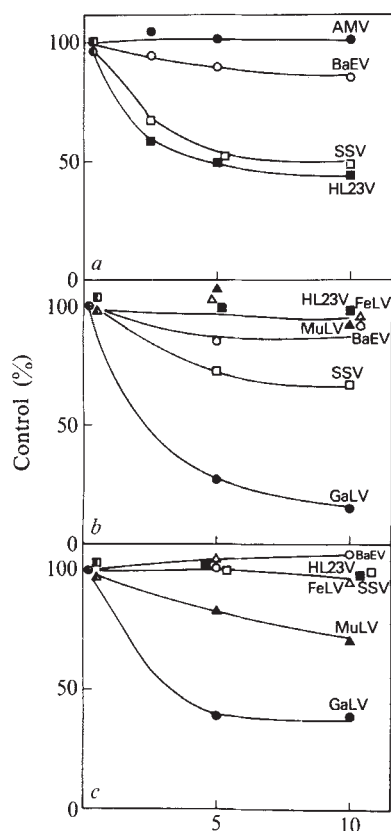


Fig. 2 Inhibition of RT purified from primate type-C viruses (SSV, GaLV, HL23V) by IgG from AML and normal leukocytes. *a*, Antibody from AML patient. DEAE Agarose-purified IgG fraction from leukocyte eluates of patient HL-80 with AML was treated overnight with pepsin bound to Sepharose beads in 0.2 M NaAC buffer, pH 3.5. The pepsin fragments were purified on an affinity column of GAH IgG (light chain specific). The purified AML F(ab')₂ fragments were then tested for inhibition of various polymerases. Cellular DNA polymerases were not inhibited (data not shown). *b*, and *c*, antibody from normal blood leukocytes. DEAE Agarose-purified IgG fractions from eluates of blood leukocytes from two different normal donors were tested against different polymerases as described in text and legend to Fig. 1. Cellular DNA polymerases were not inhibited (data not shown).

with buffy coat leukocytes (Table 2). Two typical examples are shown in Fig. 2*b* and *c*. Although RT from GaLV, and SSV are known to be closely related, the IgG from normal donors and from some AML samples (Table 1) distinguished them. At antibody concentrations of 2–5 μ g RT from GaLV was inhibited from 50% to 80% while RTs from SSV and HL23V were inhibited less than 20%.

Inhibition of RT is antibody mediated

Evidence indicates that the inhibitor of RT in eluates from some human leukocytes is a specific antibody and that the reaction studied is an antigen-antibody interaction. This evidence

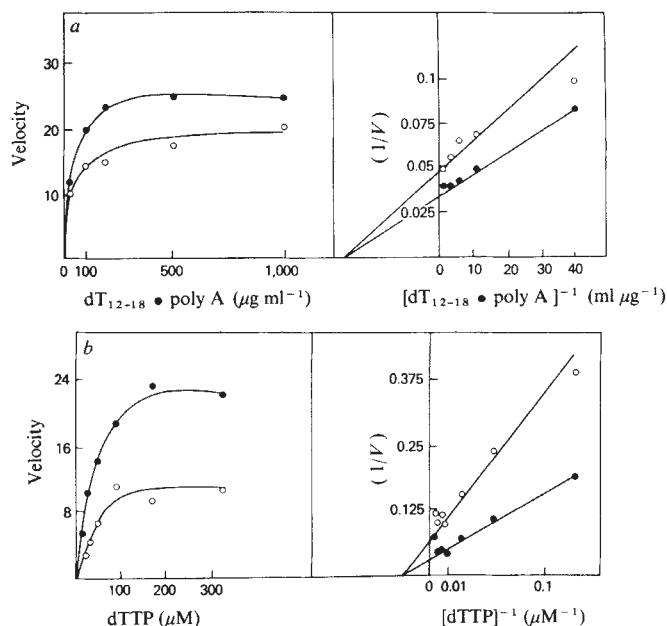


Fig. 3 Kinetics of inhibition of RT by purified IgG obtained from eluates of CML blast crisis cells (Patient HL-105). *a*, Affinity of the enzyme for the template-primer, dT₁₂₋₁₈ · poly A. Each 20- μ l reaction mixture was preincubated for 4 h at 0°C and contained 2 μ l BaEV RT, 10 mM sodium phosphate pH 8, 1% Triton X-100, 0.05% Tween 80, 130 mM NaCl, 5 mg ml⁻¹ BSA, with or without 10 μ g of IgG (purified through the DEAE agarose step) from eluates of CML patient HL-105. The enzyme activity was then assayed at 30°C for 20 min in a 200- μ l reaction mixture containing Tris-HCl pH 7.4, H³-dTTP 140 μ M (3.6 Ci mmol⁻¹), 130 μ M cold dATP, increasing amount of dT₁₂₋₁₈ · poly A (25–1,000 μ g ml⁻¹); 50 mM KCl, 0.5 mM MnCl₂. *b*, Affinity of the enzyme for substrate, dTTP. Each reaction mixture was preincubated and the enzyme assayed as in *a* except that the concentration of template-primer was constant and saturating (200 μ g ml⁻¹) and the concentration of dTTP was varied [10 μ M H³-dTTP (50 Ci mmol⁻¹) and unlabelled dTTP from 0–640 μ M]. Left panels show the velocity of the reaction (pmol dTMP incorporated into TCA precipitates in 30 min) as a function of the amount of template-primer (*a*) or dTTP (*b*) in the reaction with (○) and without (●) 10 μ g of CML antibody. Right panels show Lineweaver-Burk representation of the same data. BaEV RT was used instead of FeLV RT to avoid complete RT neutralisation. In this case the IgG cross-reacted with BeEV RT.

includes the biochemical and physical properties of the most active fraction, the specificity of inhibition of polymerisation reactions, the removal of known nonspecific inhibitors of these reactions and assays based on factors other than inhibition of enzyme activity. These are summarised below and in the following section.

The biochemical and physical properties of the most purified fraction indicate that the inhibitor is IgG as: the specific activity is increased by procedures known to purify IgG; in some instances after purification of the IgG fraction, F(ab')₂ fractions were prepared by pepsin digestion, isolated and purified by affinity chromatography using immobilised goat IgG specific for light chains. The specificity of RT neutralisation was retained in the fraction eluting as a F(ab')₂ IgG fragment (Fig. 2*a*); protein bands were observed following SDS PAGE of affinity chromatography purified IgG which coincided with IgG heavy and light chains, and the inhibitory activity was stable to heating at 56°C for 1 h and retained after dialysis.

The inhibition of RT activity is specific. Although comparable units of polymerase activity were used, positive samples showed inhibition of only certain mammalian type-C viral polymerases without inhibition of DNA polymerases from normal cells, from avian type-C viruses, or from mammalian type-B or type-D viruses. In most cases, the inhibitory activity further discriminated between RTs from various type-C mammalian viruses. The specificity was maintained with RT purified from one type of virus grown in several types of cells, for example, IgG from CML-BC comparably neutralised RT of FeLV grown in dog, cat or human cells and without neutralising RT from other viruses grown in the same cell (see legend to Fig. 1).

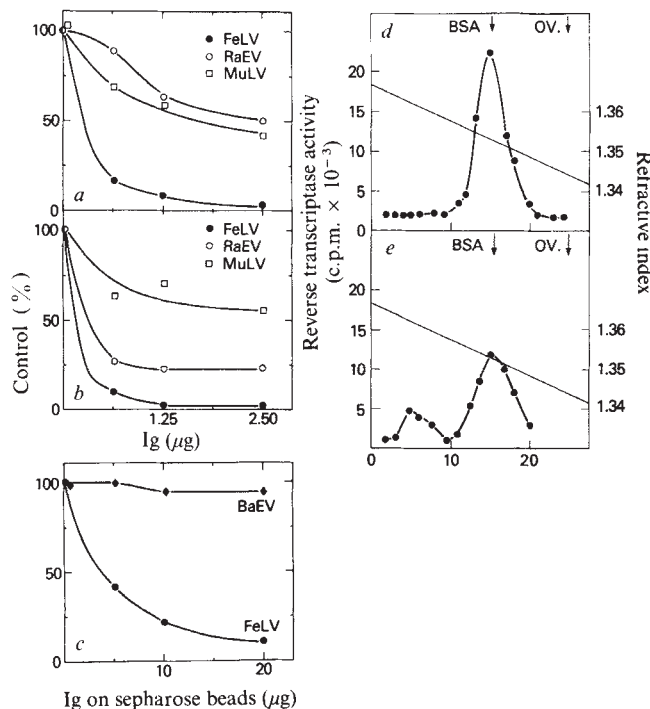


Fig. 4 Neutralisation, binding and precipitation assays for natural antibodies to mammalian type-C viral RT. Panels a–c, the IgG was the affinity chromatography purified IgG from blood leukocytes from CML blast crisis patient HL-70. *a*, Neutralisation of FeLV RT and cross-reaction with RT from rodent viruses. Other polymerases were not inhibited (see Fig. 2e). *b*, Indirect evidence for precipitation of RT from FeLV, murine leukaemia virus and rat type-C virus by IgG. Increasing amounts of IgG were preincubated with purified RT overnight at 0 °C in 40- μ l reaction mixtures constituted as described in the legend to Fig. 2. Then the samples were divided into two parts. One half was assayed directly for inhibition of RT activity as shown in *a*. The other half was centrifuged at 100,000 g for 10 min to pellet antigen–antibody complexes. The supernatant was collected and assayed for residual polymerase activity. *c*, Binding of purified FeLV RT by immobilised IgG. Control normal human IgG and IgG from patient HL-70 were immobilised on cyanogen bromide activated Sepharose 4B beads. The IgG containing beads were tested for binding of purified polymerases by mixing the polymerase with the beads in a 0.05 M Tris–HCl, pH 7.4, containing 0.3 M NaCl, 2% Triton X-100, 10 mg ml⁻¹ BSA, and 0.1% Tween 80. The mixture was incubated overnight at 0 °C, the supernatant collected after removing the beads by centrifugation, and the remaining polymerase activity in 50- μ l aliquots of the supernatant was assayed as described in the legend to Fig. 1. No polymerase bound to the control normal human IgG (data not shown) which represents 100% activity. The results are % of control RT activity remaining in the supernatant. *d*, Velocity sedimentation of purified SSV reverse transcriptase. Purified SSV RT was centrifuged at 40,000 r.p.m. for 16 h in a SW 50.1 rotor at 4 °C in a 5–20% linear sucrose gradient. Gradients were buffered with 25 mM Tris–HCl, pH 7.4, containing 10 mM sodium phosphate, 130 mM NaCl, 0.05% Tween 80 and 1% Triton X-100. Standards run simultaneously on parallel gradients were: ¹²⁵I BSA (0.03 μ g; specific activity, 75,000 c.p.m. μ g⁻¹); ¹²⁵I-goat IgG (0.025 μ g; specific activity 100,000 c.p.m. μ g⁻¹). *e*, Velocity sedimentation of SSV RT after incubation with natural antibody from a patient with CML-BC. Method is as in *d* except that the SSV RT was incubated 4 h at 0 °C with 5 μ g of purified IgG from patient HL-105. The amount of antibody used is the amount required for 70% neutralisation of SSV RT. SSV RT was used instead of FeLV RT to avoid complete enzyme neutralisation.

The purified IgG did not contain nonspecific inhibitors of RT. Inhibition of RT was not due to a protease which might degrade RT. The strongest evidence for this was the specificity of inhibition. In addition, the IgG fraction of the leukocyte eluates was tested for protease activity. Bovine serum albumin and purified RT from FeLV were labelled *in vitro* with ¹²⁵I and then incubated with either the crude leukocyte eluates, DEAE Agarose fractions or the affinity column fractions from several preparations. They were then assayed for proteases in the test IgG preparations by the release of acid soluble radioactivity or by the appearance of new radioactive peaks after velocity gradient centrifugation. As a positive control, the protein substrates were also incubated with protease K. Although the crude eluates contained protease activity, this activity was not found in

the purified IgG preparations, emphasising the need to use purified IgG. Nucleases which might hydrolyse the template-primer or phosphatases which might degrade the deoxyribonucleoside triphosphate substrate were also not involved in the inhibition of RT activity. Enzyme kinetics with certain viral polymerases (known to be inhibited by a given affinity column purified leukocyte eluate) were studied with variable concentrations of template-primer at fixed and saturating concentrations of dTTP in the presence or absence of the purified IgG (Fig. 3a). In other experiments the concentration of dTTP was varied with a fixed and saturating concentration of primer-template with and without the IgG (Fig. 3b). In both experiments the V_{max} of the enzyme was substantially decreased in the presence of the inhibitor, but there was no change in K_m . This demonstrated that the inhibition was not competitive for primer-template or for substrate, indicating that inhibition was not due to contaminating nucleases or phosphatases. Furthermore, inhibition of RT by the IgG fraction was not dependent on the type of primer-template used for polymerase activity despite their variable susceptibility to nucleases. Comparable inhibitions were obtained with dT_{12–18}·poly A, poly d(A–T), dG_{12–18}·poly C and dG_{12–18}·poly C_m (data not shown). Finally, direct assays for nuclease activities were also negative. These were performed by measuring the release of radioactivity from labelled test nucleic acids in the presence of the purified IgG. Similarly, the concentrations of Mn²⁺ and Na⁺ were varied to rule out the presence of a contaminating factor which might affect concentrations of cations optimal for RT activity. Again, no nonspecific effects were found. We conclude that the inhibitions directly involve RT.

Other assays for specific interaction of IgG with RT

When present in sufficient titre, specific antibodies against some viral structural proteins may directly precipitate the protein³⁵. Compared with radioimmune precipitation assays, enzyme neutralisation assays combined with precipitation have the advantage of being based on the identifying characteristic of RT, that is, its enzymatic function. By incubating purified RT with the IgG overnight at 0 °C and then subjecting the sample to high speed centrifugation, RT activity present in the supernatant was quantitatively removed, suggesting that RT–IgG complexes had been precipitated (Fig. 4b). This precipitation was specific to only those RTs which were shown in separate experiments to be specifically inhibited by the IgG (Fig. 4a). Figure 4a and b also show that in addition to the complete neutralisation and precipitation of FeLV RT, RT from MuLV and rat endogenous

Table 3 Abrogation by GAH IgG of the anti-RT activity present in IgG fraction purified from peripheral blood leukocyte eluates obtained from a CML blast crisis patient

Reaction mixture*	RT activity† (pmol dTMP)	% Inhibition of RT‡
Control		
RT	7.0	0
RT+GAH IgG	6.0	0
RT+goat IgG	7.3	0
Experimental		
RT+eluate antibody (Ab)	3.1	54%
RT+(eluate Ab+GAH IgG)	5.6	16%
RT+(eluate Ab+goat IgG)	3.3	50%

* Experimental design includes assays of RT activity alone and after control preincubations with GAH IgG (50 μ g) or with control goat IgG (50 μ g). RT was also preincubated with 2.5 μ g of IgG (here called eluate antibody) purified from eluate of blood leukocytes from patient HL-62 with CML (BC) either alone or in the presence of GAH-IgG or with control goat IgG. RT was purified from FeLV.

† RT activity expressed in pmol dTMP incorporated into TCA precipitates in 30 min (total c.p.m. = 30,000). Assay conditions are given in the legend to Fig. 1.

‡ % inhibition of RT were calculated from the mean value of controls.

type-C virus (RaEV) were partly affected by IgG from patient HL-70. Other viral and cellular polymerases were not affected (Fig. 1e). This specificity pattern is similar to the usual cross-reactivity of natural antibodies to FeLV RT present in sera from some FeLV-infected cats²².

The natural antibody binds specifically to type-C viral RT. Purified IgG from patient HL-70, which specifically inhibited RT from FeLV and to a lesser extent RT from rodent leukaemia viruses (Figs 1e, 4a), was tested for binding to RT as follows. The IgG was immobilised on cyanogen bromide-activated Sepharose 4B beads. The beads were then incubated with various DNA polymerases, the supernatant collected after removal of the beads and the polymerase activity remaining in the supernatant determined (Fig. 4c). FeLV RT activity was specifically removed by incubation with this immobilised IgG but not with immobilised control human IgG (Fig. 4c).

We also assayed the binding of antibodies to RT by assays dependent on the physical properties of RT activity. For this purpose, the velocity sedimentation rates in sucrose density gradients were determined for RT compared to RT preincubated with purified IgG. Concentrations of antibody (patient HL-105) were used which only partially neutralised the test RT so that some enzyme activity remained to follow the position of the RT-IgG complex in the gradient. Figure 4d shows the activity of the enzyme alone and Fig. 4e the residual activity measured through the gradient in the presence of IgG. A clear shift was observed in the enzyme activity peak to a more rapidly sedimenting peak.

The RT inhibitory activity present in the IgG can be removed by GAH-IgG. If all the inhibitory activity for viral RT were due to a specific IgG antibody reaction, the inhibitory effect of the IgG would be lessened by pretreating it with an antibody to human IgG, which was the case (Table 3). Purified FeLV RT was inhibited 55% with 2.5 µg of purified antibody fraction from leukocyte eluates obtained from CML-BC patient HL-62. When the CML antibody fraction was pretreated with goat anti-human IgG, the inhibitory effect was reduced by 75%. FeLV RT was not inhibited by GAH IgG or goat IgG.

Conclusions

The results reported here show that blood and bone marrow leukocytes carry surface proteins that can be eluted into serum free medium. Among these proteins were IgG molecules reactive with specific RTs from certain mammalian type-C oncoviruses. Among the viral RTs tested, RT from FeLV and in some instances RT from SSV, were the main DNA polymerases neutralised by IgG from blood or bone marrow cells derived from patients with myeloblastic leukaemias. The results exclude a specific interaction of the IgG with a cell protein co-purifying with viral RT as the specificity against a given RT was maintained with viruses grown in different kinds of cells from diverse species (for example, specificity of CML-BC IgG for FeLV RT with FeLV grown in either cat lymphoblasts, human lymphoblasts, or dog fibroblasts and of some AML IgG for SSV RT with SSV grown in dog thymus cells and human cells). Specificity was also maintained when different viruses were grown in the same cell (for example, a specificity of CML-BC IgG for FeLV RT when FeLV, MPMV and BaEV were grown in NC37 human lymphoblasts). Moreover, anti-RT IgG was not specifically adsorbed by uninfected cells used to grow the virus (data not shown). However, the genesis of these antibodies is unclear. It is possible that the IgG was directed against an RT which was present earlier in leukaemic and/or other cells and is now nonspecifically bound to Fc receptors on some blood cells³⁶ present in the leukaemic blood cell population, but this is unlikely in view of other data^{20,21}. It is also possible that the IgG is directed against a cell protein, mimicking mammalian type-C viral RT. This possibility cannot be excluded until the antigen is identified and chemically characterised. However, in view of the specificities shown, we think this interpretation is unlikely and our working hypothesis is that the IgG is directed against RT present on the cell membrane either as a mature protein or in the

form of a polypeptide precursor as shown with some other viral proteins³⁷ and recently also with RT precursors³⁸. In the latter study, Witte and Baltimore reported finding RT precursors on membranes of cells infected by temperature-sensitive mutants of MuLV blocked at late stages of assembly. They did not detect enzymatically active RT except when virions were formed and released. Therefore, active RT may only be made inside released, newly made virions³⁸. However, results of Wong and Kang clearly show enzymatically active viral RT in ribonucleoprotein complexes (without viral structure) present intracellularly in reticuloendotheliosis virus-transformed chicken bone marrow cells³⁹. Intracisternal as well as intracytoplasmic A-particles have been repeatedly shown to contain catalytically active RT. Our experience^{3,9-11} and that of others^{13,40} indicates that a catalytically active RT-like DNA polymerase can sometimes be identified in some fresh human leukaemic cells and also in ribonucleoprotein complexes without demonstrable virions. In some cases of AML this polymerase immunologically cross-reacted with RT from viruses of the SSV-GaLV group^{3,9-13}. These studies did not comprehensively include assays for: catalytically inactive RT, active or inactive RT in cell membranes, and FeLV or rodent virus-related RT. These studies are clearly needed in view of the recent data³⁸ and data reported here.

IgG may be associated with a specific type of cell, but this is not yet known because peripheral blood cells and bone marrow cells are heterogeneous even in the leukaemias. However, the absence of IgG with anti-RT activity from normal blood lymphocytes (purified by nylon column chromatography), the low or negative results with lymphoid leukaemia, combined with the positive results with some whole blood from normal donors and from patients with myeloid leukaemias, suggest that the antibodies are chiefly associated with non-lymphocytic cells.

There have been surprisingly few studies of antibodies against RT in animal models. One study in AKR mice indicates that such antibodies may be isolated from kidney eluates⁴¹. Serum of some animals naturally infected by retroviruses have antibodies to RT. This has recently been found in some cats infected with FeLV²², some gibbons infected with GaLV²³ and some cows infected with bovine leukaemia virus²⁴. It would be of interest to determine if anti-RT IgG can be found on the surface of leukaemic blood cells of animals, particularly from non-virus producers, as a model for the results reported here.

These results provide additional evidence for the association of type-C viral information with humans, but we do not regard them as demonstrating the presence in humans of any animal virus used here. These viruses were used to supply probes to detect antibodies which may have been directed against proteins derived from related information. For example, a number of other tests for FeLV components in humans carried out in our laboratory were negative, including tests for FeLV p30-related antigens in whole cell extracts of several of the samples used here and for anti-p30 activity in the leukocyte eluate IgG with anti-RT activity. Previous data obtained with probes derived from the available type-C viruses indicate that no human leukaemic blood cell sample tested contained complete provirus (of any of these viruses) in the majority of the cells⁴²⁻⁴⁶. Six of the CML-BC samples shown here to contain anti-RT activity against FeLV were also tested by conventional molecular hybridisation techniques and were negative for FeLV proviral sequences, providing strong evidence against active replication of FeLV or a closely related virus in these cells. On the other hand, some non-viraemic leukaemic cats do not have detectable serum antibodies to viral proteins and proviral sequences may be difficult to demonstrate⁴⁷, despite evidence for FeLV infection as suggested by the presence of feline oncornavirus membrane antigen in the tumour cells. Also, Spiegelman reported⁴⁰ small amounts of nucleic acid sequences in all human leukaemic DNA tested which were not found in DNA from normal cells, suggesting that leukaemic cells contain acquired viral sequences and some studies indicate that the extra sequences in some cases are partly homologous to sequences of

certain mammalian type-C viruses. This might be explained by the presence of incomplete provirus, by insufficient homology of the probes or by a presence of proviral sequences in a limited number of cells as described elsewhere³⁴. Therefore, it seems possible that the positive findings reported here could indicate an immunological response against the test virus used. This seems especially the case with FeLV where there is both obvious contact by humans with virus-shedding cats and known ability of the virus to infect human cells^{48,49}. The presence of antibody with relatively high activity and specificity to FeLV RT in CML-BC and the absence to date of IgG with similar anti-RT specificity in CML chronic phase, in CML in remission and in randomly selected normal blood samples might help in the detection of early blast transformation in this disease. Similarly, further distinction between IgG from AML and IgG from normal blood leukocytes characterised in the former case by a preferred reactivity against RT from SSV and in the latter case RT from GaLV_{SF}, may be useful in following patients in remission and in preleukaemic states.

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Nucleotide sequence of bacteriophage G4 DNA

G. N. Godson*, B. G. Barrell, R. Staden & J. C. Fiddes†

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

The 5,577 nucleotide long sequence of bacteriophage G4 DNA has been determined using the 'plus and minus' and chain termination methods of DNA sequencing. This sequence has been compared with that of the closely related bacteriophage ΦX174 (refs 1, 55). In the coding regions there is an average of 33.1% nucleotide sequence differences between the two genomes, but the distribution of these changes is not random and the sequence of some genes is more conserved than others. There is less sequence similarity between the untranslated intergenic regions of G4 and ΦX174, but despite this the sequences of the J/F, F/G and H/A untranslated spaces in both genomes have similar sized hairpin loops, which may be related to their function.

BACTERIOPHAGE G4 is a small isometric ΦX-like phage that was isolated in 1974 (ref. 2). Like ΦX174, it contains single-stranded circular DNA and the two phage genomes code for the same series of eleven proteins^{2,3}. However, G4 and ΦX174 do not recombine or complement efficiently⁵⁶ and electron microscopy of ΦX174/G4 DNA heteroduplex molecules indicates that there is an overall 39% base sequence mismatch between the two DNA molecules². This base sequence difference has been confirmed by the lack of similarity of the fragments obtained by restriction endonuclease digestion of ΦX174 and G4 DNA (refs 4, 5, 57). The physical maps of the two phages have, however, been aligned by detecting hybridisation between the two sets of restriction enzyme fragments^{6,56}.

The major physiological difference between ΦX174 and G4 is in the mechanism of DNA replication. Initiation and synthesis of the complementary DNA strand in ΦX174 requires the eight proteins dnaB, dnaC/D, dnaG, i, n, polIII holoenzyme and DNA binding protein^{7,8} while in G4 it requires only three proteins, dnaG, polIII holoenzyme and DNA binding protein⁹. Also, initiation of the G4 complementary DNA strand occurs at a single unique site both *in vitro*¹⁰ and *in vivo*^{11,12}, but in ΦX174 initiation of the complementary DNA strand occurs at multiple randomly located sites^{13,14}.

* Present address: Radiobiology Laboratories, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.

† Present address: Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143.

Recently, the complete nucleotide sequence of Φ X174 has been established^{1,55}. We report here the determination of the complete G4 nucleotide sequence (see Fig. 1). The G4 sequence was derived using the enzymatic priming 'plus and minus'¹⁵ and chain termination¹⁶ methods of DNA sequencing and extensive use was made of a computer to store and build up the G4 sequence and compare it with Φ X174. More detailed accounts of certain regions of the G4 genome and of the sequence determination are presented elsewhere^{3,17,58} and a description of the computer programs used has been published^{18,19}.

Structure of the G4 genome

The G4 genome is 5,577 nucleotides long, 191 nucleotides longer than the Φ X174 genome⁵⁵ and, like Φ X174, it codes for 11 genes. Four of these genes specify virion structural proteins (genes *F*, *G*, *H*, and *J*), five genes specify proteins involved in viral DNA replication and phage assembly (genes *A*, *A**, *B*, *C* and *D*), one gene specifies a lysis protein (gene *E*) and the function of the new overlapping gene *K* protein³ is not known. On SDS polyacrylamide gels (PAGE) of UV-irradiated infected

throughout the genome. A comparison of the overall structure of the G4 and Φ X174 genomes is shown in Fig. 2.

Coding regions of the G4 genome

The reading frame and starts of the G4 gene *J* and *K* coding regions³ and gene *B*, *C*, *D*, *F*, *G* and *H* coding regions⁵⁹ were established by sequencing the first 15 to 30 N-terminal amino acids of ¹⁴C-labelled G4 proteins. These proteins were isolated from SDS PAGE of UV-irradiated infected cell lysates and analysed by a radioactive microsequence method^{20,21}.

The phasing defined by the N-terminal amino acid sequences agrees with the phasing determined for the same genes in Φ X174 (ref. 1) and the N-terminal amino acid sequence of the G4 gene *C* protein clearly defines the start of the gene *C* coding region as overlapping with the termination codon of gene *A* (A-T-G-A) which was uncertain in Φ X174. The reading frames and initiation codons of the G4 *A* and *E* gene coding regions were established by analogy with Φ X174. The *A** protein is a translational restart of the *A* reading frame²² and from consideration of the size of the G4 *A** protein the start of the *A**

Table 1 Conservation of nucleotide and amino acid sequences in the coding regions of G4 and Φ X174

Gene	Codons in G4	% Amino acid changes	% Nucleotide changes	% Codon changes not changing the amino acid*	% Codons with U in the third position	
	Codons in Φ X174				G4	Φ X174
A†	554/513	37.1	36.2	25.1	30.9	39.0
A*†	341/341	21.1	22.9	28.7	29.3	36.9
B	120/120	44.2	23.9	5.8	33.3	36.7
K	56/56	39.3	23.8	7.1	25.0	26.8
C†	86/84	32.6	34.5	34.9	20.2	32.6
D	152/152	17.8	25.0	39.5	41.4	42.1
E†	96/91	46.9	23.3	5.2	15.6	14.3
J†	25/38	52.6	57.0	23.7	28.0	47.4
F	427/427	34.0	33.6	31.1	42.1	52.2
G†	177/175	60.1	51.7	21.3	39.6	56.6
H†	337/328	31.6	35.7	36.8	37.1	51.8
Total	2371/2325	34.8	33.1	27.2	33.8	43.0
A(B)‡	—	27.3	23.9	25.0	14.2	15.8
A(K)‡	—	25.0	20.5	14.3	42.9	46.4
C(K)‡	—	27.6	26.7	24.1	24.1	20.7
D(E)‡	—	16.1	20.5	33.3	40.9	39.1

The changes have been calculated from the Φ X174 sequence⁵⁵ and the G4 sequence when they have been aligned for maximum homology, and take into account deletions and insertions with respect to Φ X174. In all cases the initiation codon has been included but not the termination codon. The amino acid and nucleotide mismatches are calculated by aligning the coding regions from the ATG start and comparing the sequence, counting each deleted or inserted nucleotide as a mismatch. (For the calculations that do not include the deleted or inserted nucleotides see ref. 60.)

* Nucleotide changes in the third position of the codons and other conservative codon changes that do not change the amino acid. Results are expressed as: (the no. of conservative codon changes $\times$ 100)/total no. of codons.

† These genes have insertions and/or deletions with respect to Φ X174.

‡ Overlapping gene regions: A(B), region of gene *A* which overlaps gene *B* (A phase); A(K), region of gene *A* which overlaps gene *K* (A phase); C(K), region of gene *C* which overlaps gene *K* (C phase); D(E), region of gene *D* which overlaps gene *E* (D phase).

cells, nine of these gene products can be identified as protein bands^{2,3}. A genetic map of G4 has not been established, but a comparison of the G4 and Φ X174 nucleotide sequences indicates that the gene order in G4 is the same as in Φ X174.

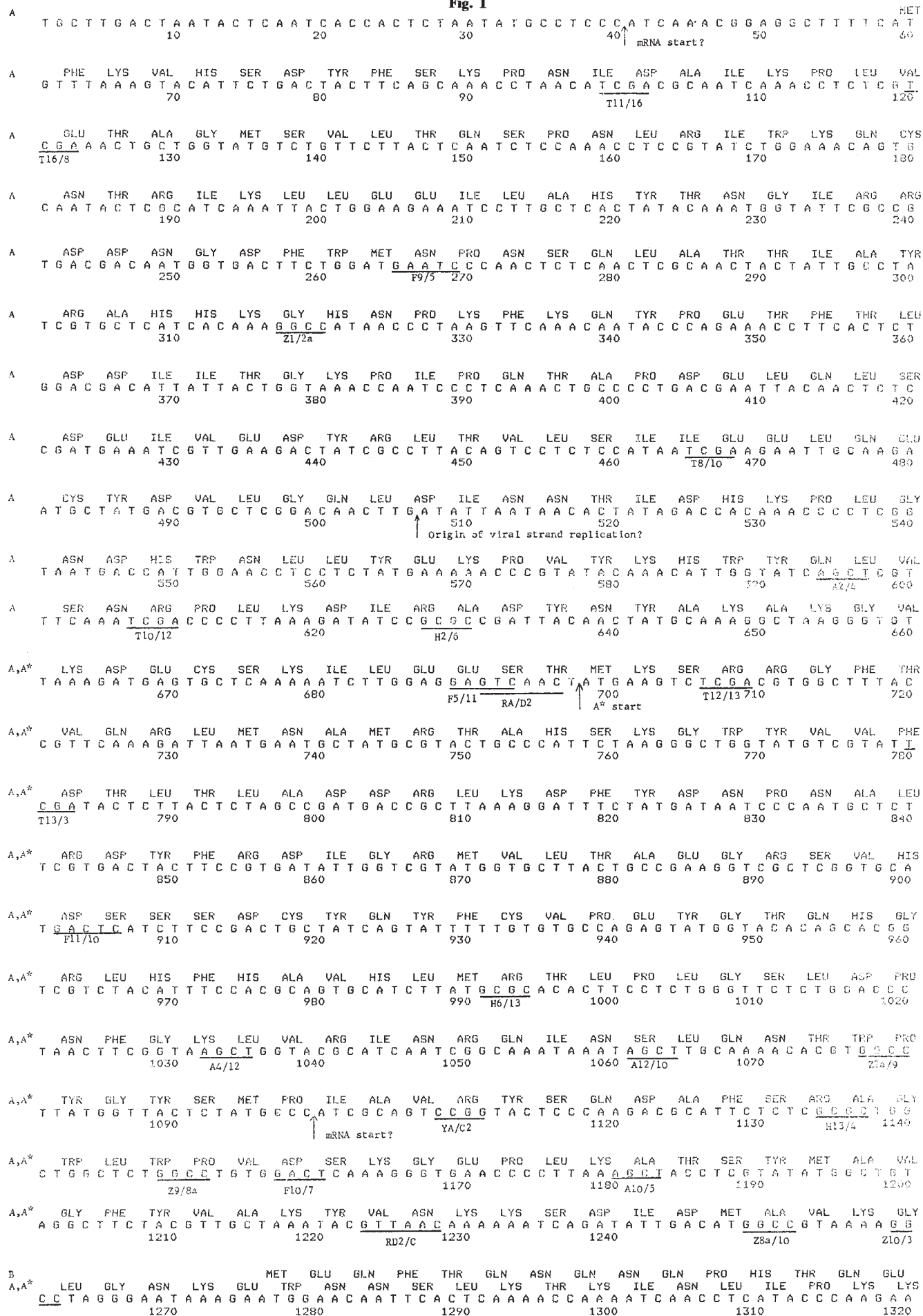
In the coding regions of G4 and Φ X174 DNA, there are enough similarities in nucleotide sequence to align the two genomes and make a gene-by-gene comparison. Some of the G4 genes are slightly larger than their Φ X174 counterparts, but only in the gene *A*, *H* and *J* coding regions are there any gross changes in size (see Table 1). The larger size of the G4 genome is mainly due to an insertion of 141 nucleotides in the G4 gene *A* coding region compared with Φ X174, increased sizes of the G4 untranslated intergenic spaces (G4 has 282 or 5.0% untranslated nucleotides and Φ X174 has 217 or 4.0% untranslated nucleotides) and duplication at the 3' end of the G4 gene *D* which introduces a 32 nucleotide long untranslated region between genes *E* and *J* that is not present in Φ X174. Also there are small insertions and deletions in G4 relative to Φ X174 elsewhere

coding region is most probably at nucleotide 698 (Fig. 1) which is an A-T-G codon preceded by a sequence complementary with the 3' end of 16S rRNA (see Table 3). A correction to the Φ X174 sequence in this region⁵⁵ agrees with this prediction.

In the coding regions, the average base sequence difference between G4 and Φ X174 is 33.1%. The distribution of these changes, however, is not random and varies from gene to gene. For instance, in the non-overlapping genes 51.7% of the nucleotides in G4 gene *G* are different from their Φ X174 counterparts, while in gene *H*, 35.7% of the nucleotides are different. In the regions of the genome containing overlapping genes, the nucleotide differences are slightly lower (see Table 1).

The amino acid differences between the G4 and Φ X174 proteins again differ with the gene. Except in the case of gene *G* protein, in which 60.1% of the G4 amino acids are different from their Φ X174 equivalent, the differences in amino acids between the G4 and Φ X174 virion structural proteins (*F*, *G*, *H* and *J*) is no greater than in the non-virion structural proteins *A*,

Fig. 1



B
A₁* SER VAL GLN ASN THR ASN VAL SER GLN PHE ARG ASN GLU THR VAL ILE ASN GLY SER PRO
VAL PHE ARG ILE ARG MET SER ARG ASN PHE GLY MET LYS LEU SER MET ALA HIS LEU
A G T G T T C A G A T A C G A A T G T C T C G C A A T T T C G G A A T G A A A C T G T T A T C A A T G G C T C A C C T
1330 1340 1350 1360 1370 1380

B
A₁* VAL SER GLY ASN PRO ASP GLY THR ASP PRO SER GLY LEU ARG ARG ASP PRO VAL GLN GLN
SER ALA GLU THR LEU MET GLU LEU THR GLN VAL GLY TYR ASP VAL THR PRO PHE ASN ALA
G T C A G C G G A A C C C T G A T G G A A C T G A C C C A A G T G G G T T A C G A C G T G A C C C G T T C A C A A
1390 1400 1410 1420 1430 1440

B
A₁* HIS LEU GLU ALA GLU ARG GLN LYS ARG ALA GLN ILE GLU ALA GLY LYS GLU ILE CYS ARG
ILE LEU LYS GLN ASN ALA LYS LYS GLU LEU LYS SER ARG LEU ALA LYS LYS SER VAL ALA
C A T C T T G A A G C A G A A C G C C A A G A A A G A G C T C A A A T C G A G G C T G G C A A A G A A T C T G T C G C
1450 1460 A5/1 T3/9 1480 1490 1500

B
A₁* ARG ARG PHE LEU GLY GLY ALA THR CYS ASP ASP GLU SER LEU ALA LYS ILE HIS ALA GLN PHE ASP
ASP VAL LEU GLU ALA GLN PRO VAL THR THR ASN LEU LEU LYS PHE MET ARG ASN LEU THR
C G A C G T T T G G A G G C G C A A C C T G T G A C G A T C G A A T C T G C T A A A T T C A T G C G C A A T T T G A C
1510 H4/15 1520 1530 F7/3 1540 H15/3 1560

B
A₁* PRO ASN ASN ARG SER VAL GLN PRO THR GLU PHE TYR ARG PHE ASN ASP HIS GLU ILE ASN
ARG THR ILE GLY ALA SER ASN LEU GLN SER PHE ILE ALA SER MET THR THR LYS LEU THR
C C G A A C A T C G G A G C G T C C A A C C T A C A G A G T T T T A T C G C T T C A A T G A C C A C G A A A T T A C
1570 1580 1590 1600 1610 1620

B
A₁* LYS TYR GLY TYR PHE ***
SER THR ASP ILE SER ASP MET GLU THR LYS LYS ASN TYR VAL ALA SER ALA GLY ILE ALA VAL ALA
K A A G T A C G G A T A T T T C T G A T G A A A C C A A A A C T A C G T T G C T T C T G C A G G A A T T G C T G T T G C
1630 1640 1650 1660 P1/1 1670 1680

C
A₁* ASN LEU ARG ILE LYS SER LYS TRP THR ALA GLY GLY LYS ***
K THR TYR GLU LEU ASN ARG SER GLY LEU LEU VAL GLU ASN GLU GLU ILE GLN SER GLN LEU
T A A C T T A C G A A T T A A A T C G A A G T G G A C T G C T G G T G G A A A T G A G G A A A T T C A A T C T C A A C
1690 T9/14 1710 1720 1730 1740

C
K LEU LYS ASN SER ARG SER SER TYR PHE ALA THR PHE ARG HIS HIS LEU ASN VAL LEU ALA
LYS LYS LEU GLU VAL LEU LEU CYS ASN LEU SER PRO SER SER GLN ARG ALA GLY LYS
T T A A A A A C T C G A G G T C T T A C T T T G C A A C C T T T G C C A T C A T C T C A A C G T G C T G G C A
1750 T14/6 1760 1770 1780 1790 1800

C
K LYS THR ASP ALA LEU ASP GLU GLU LYS TYR LEU ASN MET LEU GLY ALA LEU LEU LYS ASP
ASN ***
A A A A C T G A C G C C C T T G A C G A G G A A A T A C T T A A A T A T G T T A G G T G C T C T C C T C A A G G A C
1810 1820 1830 1840 1850 1860

C
K TRP PHE ARG TYR GLU GLU HIS PHE VAL HIS GLY LYS GLN SER MET LEU ASP ILE LEU LYS
T G G T T C C G G T A C G A A G A A C A T T T C G T G C A T G G T A A C A A T C A A T G C T T G A C A T A C T G A A A
YC2/D 1880 1890 1900 1910 1920

C
D GLU ARG GLY LEU LEU SER THR SER SER THR ASP THR ASN HIS LYS GLY ASN ***
G A A C G T G G C C T A T T A T C C A C A T C G T C A A C T G A C A C A A A C C A C A A G G A A A C T G A A A T G T C
23/2b 1940 RC/E 1950 1960 1970 MET SER
mRNA start?

D
D LYS SER ASN GLU SER ALA VAL ALA PHE GLN THR ALA ILE ALA SER ILE LYS LEU ILE GLN
T A A A T C A A A C G A A T C T G C T G T A G C C T T T C A A A C T G C T A T C G C T T C T A T C A A A C T T A T T C A
1990 F3/1 2000 2010 2020 2030 2040

D
D ALA SER SER VAL LEU ASP LEU THR GLU ASP ASP PHE ASP PHE LEU THR ARG ASP ARG VAL
A G C A T C T T C T G T T C T T G A C T T G A C A G A G G A C G A T T T T G A C T T C T T A A C T C G C G A C C G T G T
2050 2060 2070 2080 2090 2100

D
E TRP ILE ALA THR ASP ARG SER ARG ALA ARG ARG ALA ILE GLU ALA CYS VAL TYR GLY THR
C T G G A T T G C T A C C G A C C G T T C A C G C G C T C G C G T G C T A T C G A G G C T T G C G T A T G G A A C
2110 H3/8 2130 T6/4 2150 2160

D
E LEU ASP PHE VAL GLY TYR PRO ARG PHE PRO ALA PRO VAL GLU PHE ILE SER ALA VAL ILE
TRP THR LEU SER GLY ILE LEU ALA PHE LEU LEU LEU LEU SER LEU PHE LEU PRO SER LEU
A C T G G A C T T T G T C G G G T A T C C T C G C T T T C A A A C T G C T C T G T T G A G T T T A T T T C T G C C G T C A T
2170 2180 2190 2200 2210 2220

D
E ALA TYR TYR VAL HIS PRO VAL ASN ILE GLN THR ALA PRO CYS LEU ILE MET GLU GLY ALA GLU
LEU ILE THR PHE ILE PRO LEU THR SER LYS PRO VAL SER SER TRP LYS VAL LEU SER
T G C T T A T T A C G T T C A T C C C G T T A A C A T C C A A A C C G C C T G T C T C A T C A T G G A A G G T G C T G A
2230 2240 RE/D1 2250 2260 2270 2280

D
E PHE THR GLU ASN ILE VAL ASN GLY VAL GLU ARG PRO VAL LYS ALA SER GLU LEU PHE ALA
LEU PRO LYS THR SER SER MET VAL LEU ASN ALA PRO LEU LYS LYS PRO LEU ASN CYS SER PRO
G T T T A C C G A A A C A T C G T C A A T G G T G T T G A A C G C C C G T T A A A G C C T C T G A A C T G T T C G C
2290 2300 2310 2320 2330 2340

D
E PHE THR LEU LEU VAL ARG ALA GLY ASN LYS ASP LEU ILE GLY HIS ALA GLU THR ASN ILE
SER LEU PHE LEU PHE ALA PRO GLU THR LYS ILE LEU SER VAL THR LEU LYS GLN THR SER
C T T C A C T C T T C T T G T T C G C G C C G G A A C A A A G A T C T T A T C G G T C A C G C T G A A A C A A C A T
2350 H8/14 YD/C1 2370 MB/A 2380 2390 2400

D
E ARG GLU GLN LEU ARG ALA GLN GLY VAL MET ***
VAL ASN SER TYR ALA LEU LYS VAL SER CYS LYS ASP LEU ***
C C G T G A A C A G T T A C G C G C T C A A G G T G T C A T G T A A A G A C C T T T G A T T T T A T C G T C T T C A C T
2410 H14/7 2420 2430 2440 2450 2460

J T T T A A G G A G T T A T G T A A T G A A A A A T C A A T T C G C C G C T C T G G T G G C A A A T C T A A G G G T G C
2470 2480 2490 2500 2510 2520

J ARG LEU TRP TYR VAL GLY GLY THR GLN TYR ***
C C G T C T C T G G T A T G T A G G C G G A A C A C A A T C T A A T C T T T A T G T G G G A C C G C G G T C C C A C
2530 2540 2550 2560 2570 2580

F MET SER ASN VAL GLN THR SER ALA ASP ARG VAL PRO HIS ASP
T C T A T T T A A G G A T A C A A A A A T G T C T A A C G T T C A A A C A T C T G C G G A C C G T G T A C C T C A T G A
2590 2600 2610 2620 2630 2640

F LEU SER HIS LEU VAL PHE GLU ALA GLY LYS ILE GLY ARG LEU LYS THR ILE SER TRP THR
C T T A T C T C A C C T T G T C T T T G A G G C T G G T A A A A T T G G C C G C C T C A A A A C T A T C T C T T G G A C
2650 2660 2670 22b/8b 2680 2690 2700

F PRO GLY GLY ALA GLY ASP SER PHE GLU CYS ASP MET VAL GLY ALA ILE ARG LEU SER PRO
G C C T G G T G G T G C T G G T G A C T C T T T C G A G T G T G A T A T G G T T G G C G C T A T T C G T C T G T C C C C
2710 F1/19 T4/1 2730 2740 H7/5 2750 2760

F LEU ARG ARG GLY LEU ALA VAL ASP SER ARG VAL ASP ILE PHE PRO PHE TYR ILE PRO HIS
T C T T C G T C G T G G C C T C G C T G T T G A C T C A C G C G T T G A T A T C T T T C C T T T C T A T A T C C C A C A
2770 28b/5b 2780 RD1/B F19/16 2790 2800 2810 2820

F ARG HIS ILE TYR GLY GLN GLN TRP ILE ASN PHE MET LYS ASP GLY VAL ASN ALA SER PRO
C C G T C A T A T C T A C G G T C A G C A G T G G A T T A A C T T T A T G A A G A T G G C G T T A A T G C C T C C C C
2830 2840 2850 2860 2870 2880

F LEU PRO PRO VAL THR CYS SER SER GLY TRP ASP SER ALA ALA TYR LEU GLY THR ILE PRO
T C T T C C T C C T G T T A C A T G C T C C T C T G G T T G G G A C T C T G C T G C T A T C T C G G T A C C A T T T C
2890 2900 2910 F16/2 2920 2930 K1/1 2940

F SER SER THR LEU LYS VAL PRO LYS PHE LEU HIS GLN GLY TYR LEU ASN ILE TYR ASN ASN
G T C T T C T A C C C T T A A A G T G C C T A A A T T C T T A C A T C A G G G C T A T C T G A A T A T T A T A T A A
2950 2960 2970 2980 2990 3000

F TYR PHE LYS PRO PRO TRP SER ASP ASP LEU THR TYR ALA ASN PRO SER ASN MET PRO SER
C T A C T T C A A A C C G C C T T G G T C T G A T G A C T T A A C T T A C G C T A A C C C A T C C A A T A T G C C T T C
3010 3020 3030 3040 3050 3060

F GLU ASP TYR LYS TRP GLY VAL ARG VAL ALA ASN LEU LYS SER ILE TRP THR ALA PRO LEU
T G A G G A T T A T A A A T G G G G C G T A C G T G T C G C T A A C C T T A A A T C T A T C T G G A C T G C G C C A C T
3070 3080 3090 3100 3110 H5/10 3120

F PRO PRO ASP THR ARG THR SER GLU ASN MET THR THR GLY THR SER THR ILE ASP ILE MET
T C C A C C G G A T A C T C G T A C A T C T G A A A A C A T G A C T A C T G T A C A T C C A C T A T T G A C A T T A
3130 YC1/B 3140 3150 3160 3170 3180

F GLY LEU GLN ALA ALA TYR ALA LYS LEU HIS THR GLN GLU ARG ASP TYR PHE MET THR
G G C C T T C A A G C C G C A T A C G C T A A A T T A C A T A C G G A A C A G G A A C G T G A T T A C T T C A T G A C
3190 3200 3210 3220 3230 3240 25b/4

F ARG TYR ARG ASP ILE MET LYS GLU PHE GLY GLY HIS THR SER TYR ASP GLY ASP ASN ARG
C C G T T A C C G T G A C A T T A T G A A A G A G T T C G G C G G T C A T A C A T C C T A T G A T G G T G A C A A T C G
3250 3260 3270 3280 3290 3300

F PRO LEU LEU LEU MET ARG SER GLU PHE TRP ALA SER GLY TYR ASP VAL ASP GLY THR ASP
T C C T C T T C T G C T C A T G C G C T C T G A A T T T T G G G C A T C T G G C T A T G A C G T A G A C G G T A C T G A
3310 3320 H10/1 3330 3340 3350 3360

F GLN SER SER LEU GLY GLN PHE SER GLY ARG VAL GLN GLN THR PHE ASN HIS LYS VAL PRO
C C A A T C T T C T C T C G G T C A A T T C T C T G G T C G C G T T C A G C A G A C C T T C A A T C A C A A A G T C C C
3370 3380 3390 3400 3410 3420

F ARG PHE TYR VAL PRO GLU HIS ARG VAL ILE MET THR LEU ALA VAL THR ARG PHE PRO PRO
A C G C T T C T A T G T T C C T G A G C A T C G C G T A A T T A T G A C T C G G C G G T T A C T C G C T T T C C G C C
3430 3440 3450 F2/13 3460 3470 3480

F THR HIS GLU MET GLU MET HIS TYR LEU VAL GLY LYS GLU ASN LEU THR TYR THR ASP ILE
T A C T C A T G A A A T G G A A T G C A C T A T C T T G T A G G T A A A G A A A C T T A A C T A T A C C G A T A T
3490 3500 3510 3520 3530 3540

F ALA CYS ASP PRO ALA LEU MET ALA ASN LEU PRO PRO ARG GLU VAL SER LEU LYS GLU PHE
T G C T T G T G A C C C T G C A C T G A T G G C T A A C C T G C C A C C T C G T G A A G T A T C C T T A A A A G A A T T
3550 3560 3570 3580 3590 K11/1 3600

F PHE HIS SER SER PRO ASP SER ALA LYS PHE LYS ILE ALA GLU GLY GLN TRP TYR ARG THR
C T T C C A C T C C T C A C C C G A T T C T G C T A A A T T C A A A A T C G C T G A G G G C C A A T G G T A T C G T A C
3610 F13/18 3630 3640 24/7b 3650 3660

F GLN PRO ASP ARG VAL ALA PHE PRO TYR ASN ALA LEU ASP GLY PHE PRO PHE TYR SER ALA
A C A G C C T G A C C G T G T C G C T T C C A T A T A T G C T C T G A T G G A T T C C C G T T C T A C T C T C
3670 3680 3690 F18/14 3700 3710 3720

F LEU PRO SER THR GLU LEU LYS ASP ARG VAL LEU VAL ASN THR ASN ASN TYR ASP GLU ILE
T C T C C C G T C C A C G G A A C T A A A A G A C C G T G T A C T G G T T A A C A C T A A T A A C T A C G A T G A A T
3730 3740 3750 RB/A 3770 3780

F PHE GLN SER MET GLN LEU ALA HIS TRP ASN MET GLN THR LYS PHE ASN ILE ASN VAL TYR
 C T T C C A G T C T A T G C A G C T T G C A C A C T G G A A T A T G C A A A C T A A A T T A A C A T T A A C G T T T A
 3790 A1/7 3800 3810 3820 3830 3840

F ARG HIS MET PRO THR THR ARG ASP SER ILE MET THR SER ***
 T C C T C A C A T G C C T A C G A C A C G T G A C T C A A T C A T G A C C T C G T A A C G C A A C A A A G S C C G C C C
 3850 3860 F14/17 3870 3880 3890 27b/5a 3900

C T C T A C T G G T C A G A T A C C T G C C C A A T G T G G G G C G G A C C G T G C C T A C G G A S A T A C T C G A G T
 3910 3920 3930 3940 3950 T1/5 F17/4

G C T C C G A T A C A T G G A C G G C G A A A G C C G C C G T C C C T A C T G C A A A G C C A A A A G G A C T A A C A T A MET
 3970 3980 RNA PRIMER 3990 4000 4010
 Origin of complementary strand synthesis

G PHE GLN LYS PHE ILE SER LYS HIS ASN ALA PRO ILE ASN SER THR GLN LEU ALA ALA THR
 T G T T C C A G A A A T T C A T T T C T A A G C A C A A T G C T C C A A T T A A C T C T A C T C A G C T T G C T G C T A
 4030 4040 4050 4060 A7/13 4080

G LYS THR PRO ALA VAL ALA ALA PRO VAL LEU SER VAL PRO ASN LEU SER ARG SER THR ILE
 C T A A A A C C C C A G C T G T A G C G G C A C C T G T T T T A T C T G T G C C T A A C T T A A G T C G C T C T A C T A
 4090 A13/3 4100 4110 4120 4130 4140

G LEU ILE ASN ALA THR THR THR ALA VAL THR THR HIS SER GLY LEU CYS HIS VAL VAL ARG
 T T C T A A T C A A T G C A A C A A C C A C A C C G T T A C A A C T C A T T C A G G C T T G T G C C A T G T T G T T C
 4150 4160 4170 4180 4190 4200

G ILE ASP GLU THR ASN PRO THR ASN HIS HIS ALA LEU SER ILE ALA GLY SER LEU SER ASN
 G C A T T G A T G A A C A A A C C C A A C A A A T C A T C A C G C T C T A T C T A T T G C A G G T T C G T T A T C A A
 4210 4220 4230 4240 4250 4260

G VAL PRO ALA ASP MET ILE ALA PHE ALA ILE ARG PHE GLU VAL ALA ASP GLY VAL VAL PRO
 A C G T C C C T G C T G A T A T G A T T G C T T T T G C C A T T C G T T T T G A G G T C G C T G A T G G C G T C G T T C
 4270 4280 4290 4300 4310 4320

G THR ALA VAL PRO ALA LEU TYR ASP VAL TYR PRO ILE GLU THR PHE ASN ASN GLY LYS ALA
 C T A C T G C C G T C C C G G C C T T A T A C G A C G T C T A T C C A A T C G A A A C C T T C A A T A A C G G C A A A G
 4330 YB/E 25a/6 4340 4350 T5/15 4370 4380

G ILE SER PHE LYS ASP ALA VAL THR ILE ASP SER HIS PRO ARG THR VAL GLY ASN ASP VAL
 C A A T C T C A T T T A A G A T G C T G T T A C C A T C G A C T C A C A T C C C C G C A C C G T C G G T A A C G A C G
 4390 4400 T15/2 F4/20 4420 4430 4440

G TYR ALA GLY ILE MET LEU TRP SER ASN ALA TRP THR ALA SER THR ILE SER GLY VAL LEU
 T T T A T C C G G G A A T C A T G C T C T G G T C T A A C G C T T G G A C C G C T C T A C T A T C T C T G G C G T C
 4450 F20/12 4460 4470 4480 4490 4500

G SER VAL ASN GLN VAL ASN ARG GLU ALA THR VAL LEU GLN PRO LEU LYS ***
 T C T C T G T T A A T C A G G T A A C C G T G A A G C A A C C G T C C T T C A A C C T C T G A A A T A A G G A T T A T
 4510 4520 4530 4540 4550 4560

H MET PHE GLY SER ILE ALA GLY GLY ILE ALA SER ALA LEU ALA GLY GLY LEU MET GLY
 C C T A T G T T T G G C T C T A T C G C T G G C G G T A T C G C C T C C G C A C T T G C C G G A G G C C T T A T G G G T
 4570 4580 4590 4600 YE/F 26/7a 4620

H LYS LEU PHE GLY GLY GLY GLN SER ALA ASP SER THR GLY ILE GLN GLY ASN VAL LEU ALA
 A A A T T A T T T G G C G G C G G T C A G T C C G C C G A C T C T A C C G G A A T C C A A G G C A A C G T C C T T G C T
 4630 4640 F12/21 YF/A F21/15 4670 4680

H SER ASP ASN ASN VAL VAL GLY ALA ASN ASP ALA GLY ILE LYS SER ALA ILE GLN GLY SER
 T C C G A C A A C A A T G T T G T A G G T G C T A A T G A T G C T G G T A T T A A A T C T G C T A T T C A G G G C T C T
 4690 4700 4710 4720 4730 4740

H THR VAL PRO ASN SER GLN GLU ALA ALA PRO SER ALA ILE SER GLY ILE LEU ALA ASP SER
 A C T G T C C C T A A C T C T C A G G A A G C T G C T C C C T C T G C T A T C T C T G G C A T C C T T G C C G A C T C T
 4750 4760 A3/8 4770 4780 4790 F15/8 4800

H GLY LYS HIS ALA LEU SER SER LEU THR ASN ALA GLY ALA ASN LYS LEU MET GLU ALA VAL
 G G C A A G C A T G C G C T C T C G T C T T A C C A A T G C T G G T G C A A A T A A A C T C A T G G A G G C T G T C
 4810 H1/12 4820 4830 4840 4850 4860

H GLY LEU SER LYS SER ALA SER ASP LYS GLY LYS ASP THR LYS ASP TYR LEU ALA ALA
 G G C C T T T C T A A G T C T G C C T C T G A C A G G G C A A G G A C A A A G A C T A T C T C G C C G C C G C A
 27a/1 4870 4880 4890 4900 4910 4920

H PHE PRO GLU LEU ASN PRO TRP GLU ARG ALA GLY ALA GLY ALA SER SER PRO GLY MET GLN
 T T C C C C G A G C T C A A C C C A T G G G A A C G T G C T G G T G C T G G C G C T T C T A G T C C A G G C A T G C A A
 4930 4940 4950 H12/9 4970 4980

H ASP ALA GLY PHE GLN ASN GLN LYS GLU LEU THR LYS MET GLN LEU ASP ASN GLN LYS GLU
 G A C G C T G G C T T C C A A A A T C A G A A G G A G C T A A C C A A G A T G C A G C T T G A C A A C C A A A A G G A A
 4990 5000 A11/14 5020 A14/6 5030 5040

H ILE ALA LYS MET GLN ASN GLU THR GLN LYS GLU ILE ALA GLY LEU GLN SER ILE THR SER
 A T C G C T A A G A T G C A A A A C G A A A C T C A G A A G G A A A T T G C T G G G C T T C A A T C T A T T A C A T C A
 5050 5060 5070 5080 5090 5100

H	ARG	GLU	ASN	THR	LYS	ASP	THR	VAL	TYR	ALA	GLN	ASN	GLU	MET	LEU	ALA	TYR	ASN	GLN	LYS	
	C	G	C	G	A	A	A	T	A	C	T	A	A	G	A	T	A	C	C	A	A
			5110				5120			5130			5140			5150				5160	
H	GLU	SER	MET	SER	ARG	VAL	GLY	ALA	ILE	LEU	GLU	ASN	THR	SER	LEU	THR	LYS	GLN	GLN	GLN	
	G	A	G	T	C	T	A	T	G	T	C	A	A	A	C	A	C	A	A	A	
	F8/6		5170				5180		H9/11				5200			5210				5220	
H	THR	SER	GLU	ILE	MET	ARG	GLN	MET	LEU	THR	GLN	ALA	GLN	THR	ALA	GLY	GLN	TYR	PHE	THR	
	A	C	T	T	C	T	G	A	A	T	T	G	C	T	C	A	A	T	T	T	
		5230					5240			5250			5260			5270				5280	
H	ASN	ASP	GLN	ILE	LYS	GLU	LEU	THR	ARG	LYS	VAL	GLY	ALA	ASP	ILE	ASP	ALA	VAL	ARG	ALA	
	A	A	T	G	A	C	C	A	A	T	C	A	A	G	G	T	G	C	T	G	
		5290					A6/9			5310			5320			5330			H11/2		
H	ASN	THR	ALA	ARG	THR	HIS	VAL	GLU	THR	ASP	ARG	SER	LYS	GLN	GLU	VAL	GLN	ASN	SER	ARG	
	A	A	C	A	C	T	G	C	A	C	C	G	C	T	C	A	A	A	C	C	
		5350					5360			5370			5380			5390				5400	
H	TYR	ALA	SER	SER	GLN	VAL	GLY	LYS	THR	ALA	LYS	ASP	VAL	SER	ASN	ALA	ILE	THR	ASP	THR	
	T	A	T	G	C	C	T	C	T	T	C	A	G	G	T	G	T	C	C	A	
		5410					5420			5430			5440			5450				5460	
H	ALA	GLY	SER	ILE	VAL	ASP	TYR	PHE	ARG	GLY	ALA	ASP	GLN	LYS	VAL	ALA	ASP	VAL	TYR	ASN	
	G	C	T	G	G	T	T	C	T	A	T	T	T	C	G	T	G	G	T	T	
	A9/2		5470				5480			5490		NA/B	5500			5510				5520	
H	ASN	TYR	PHE	LYS	ASP	GLY	LYS	SER	LYS	GLY	ILE	GLU	SER	ASN	HIS	ARG	SER	LYS	***		
	A	A	C	T	A	T	T	C	A	A	G	A	T	G	A	T	C	C	A	A	
		5530					5540		T7/11	5550		F6/9	5560			5570					

Fig. 1 Nucleotide sequence of bacteriophage G4 wild-type DNA. The sequence of the circular DNA molecule is written starting after the termination codon for the *H* gene and is numbered accordingly. The letters in the left hand margin indicate the protein whose amino acid sequence is shown in the corresponding line. Restriction enzyme recognition sites are underlined. The single letter code used for the enzymes is as follows: A, *AluI*; F, *HinfI*; H, *HhaI*; K, *KpnI*; M, *MboI*; P, *PstI*; R, *HindII*; RI, *EcoRI*; T, *TaqI*; Y, *HpaII*; Z, *HaeIII*. The G4 promoters and origins of DNA replication are marked accordingly. Nucleotides 87, 91, 1123, 2,780–2,790, 2952, and 4,096–4,098, are tentative. The amino acids are those predicted from the nucleotide sequence.

B, C, D, E and K which are presumed to have an enzymatic function in synthesis and assembly of the virus. The amino acid differences between the proteins of the two phages in Table 1 are the most highly conserved protein between the two phages, with only 17.8% of the amino acids different due to the presence of the overlapping *E* gene within the gene *D* coding region (see below). The amino acid differences do not always correlate with the nucleotide differences of the coding region. This is because in some genes a high percentage of nucleotide differences are in the third degenerate position of the codon which conserves the amino acid sequence (compare gene *D* with gene *G* in Table 1).

Overlapping genes

Two overlapping gene systems have been identified in Φ X174 in which the nucleotide sequence is read in two different phases. These are gene *E* which is entirely coded within the gene *D* coding region²³ and gene *B* which is entirely coded within the gene *A* coding region^{24,25}. Both of these overlapping gene systems are present in G4. The G4 gene *B* coding region has been identified by sequencing the 20 N-terminal amino acids of the G4 protein²⁹ and it is contained within G4 gene *A* coding region, exactly as in Φ X174. Gene *E* protein has not yet been identified on SDS PAGE either in Φ X174 or G4 and the location of the gene *E* coding region in the G4 genome has been deduced from similarities with the Φ X174 sequence (that is, the conservation of a gene *E* ribosome binding site sequence, the A-T-G initiation codon and the characteristic hydrophobic N-terminal amino acids). The G4 *E* gene coding region starts in the same position as the Φ X174 *E* gene, but it is five codons longer and, unlike Φ X174, the G4 *E* gene coding region extends beyond the end of the *D* gene (see Fig. 2). A third overlapping gene system has been identified in G4 (ref. 3). This is gene *K* which is coded as a second phase of genes *A* and *C*, crossing the junction of those two coding regions (see Fig. 2). This overlap-

ping gene system was identified by isolating the G4 *K* protein and determining part of its amino acid sequence. G4 gene *K* is of particular interest because five nucleotides of its coding region are read as a third phase of pre-existing overlapping genes (see Figs 1 and 2).

The nucleotide differences between the overlapping gene regions of the G4 and Φ X174 genomes are shown in Table 1. The frequency of changed nucleotides in the G4 gene *B*, *K* and *E* coding regions compared with the same regions in Φ X174 are 23.9%, 23.8% and 23.3%, respectively. This is approximately 10–20% below the frequencies for the non-overlapping genes. However, in the three overlapping genes, fewer of the nucleotide changes are third position or conservative codon changes and this results in the gene *B*, *K* and *E* proteins having a higher than average amino acid difference between the two phages. It would appear, therefore, that there is considerable selective pressure to preserve the gene *A*, *C* and *D* amino acid sequence.

This agrees with the suggestion made previously²³ that gene *E* in Φ X174 arose at a later stage of evolution than the *D* gene and was a result of the phage utilising the high incidence of *D* phase codons ending in T to generate a second phase protein which was hydrophobic, as codons with T in the second position specify hydrophobic amino acids. It may be inconsistent, however, with the suggestion²⁴ that the present day overlapping *A* and *B* genes are a result of an evolutionary readthrough of a separate *A* gene into a pre-existing *B* gene coding region.

The most noticeable feature of the gene *E* and *K* proteins is their strongly hydrophobic N terminus. Both G4 gene *E* and *K* proteins have 10 leucine residues in the first 25 N-terminal amino acids and these are distributed in two blocks in a manner similar to certain secretory precursor proteins^{3,26}. This suggests that the most important feature to preserve in these proteins is their hydrophobic N terminus and the amino acid sequence of the rest of the molecule is more free to drift in evolution.

Thus in the overlapping coding regions of G4 and Φ X174, the frequency of nucleotide change is only slightly lower than in the non-overlapping coding regions and the increased number of amino acid changes in the overlapping gene proteins appears to be accommodated by the lack of stringency in the amino acid sequence. Use of the DNA sequence in two concurrent reading frames need not, therefore, be a significant barrier to evolutionary change.

Codon use

G4 DNA does not have the same high T content as Φ X174 DNA and its base composition as obtained from the nucleotide sequence is A, 27.17%, C, 25.93%, G, 19.80%, and T, 27.11%. This compares with the Φ X174 base composition of A, 23.95%, C, 21.48%, G, 23.30% and T, 31.27% (ref. 55). The high frequency of codons with T in the third position that was noted in Φ X174 (ref. 27) is not so marked in G4. Overall, 33.8% of the G4 codons have T in the third position compared with 43.0% in Φ X174. A gene-by-gene analysis of the number of codons with third position T is shown in Table 1. It is noticeable that in the overlapping gene regions of the genome, the percentage of codons with T in the third position is high and almost the same in both G4 and Φ X174, but in the non-overlapping gene regions which code for the structural virion proteins, although the incidence of third position T is still high in Φ X174, it is much lower in G4.

The spectrum of codon use in G4 is similar to that in Φ X174. This is shown in Table 2 and is compared with the codon use in MS2, which is the only prokaryotic virus for which extensive information is available²⁸. Some codons are rarely used in G4. For example, out of a total of 123 Arg codons, only 4 and 6 are A-G-G and A-G-A respectively, and out of 139 Gly codons, only five are G-G-G. The figures are similar in Φ X174. In general, codons ending in G-G and G-A are rare in both G4 and Φ X174. These rare codons may have a modulating role in translation if their corresponding tRNA is also rare, as suggested by Fiers *et al.*²⁸. The use of codons by these three prokaryotic viruses is considerably different from the use of codons by the eukaryotic virus SV40 (refs 29, 30).

Most of the G4 and Φ X174 genes have the same termination codons, except for genes *F* and *G*, which use T-A-A in G4 and T-G-A in Φ X174. The A-T-G-A overlapping termination codon of gene *A* and initiation codon of gene *C* is present in both genomes, but the two other overlapping Φ X174 termination and initiation codons (genes *C/D* and *D/J*) are not present in G4.

Untranslated regions

There are untranslated sequences between most of the G4 coding regions and two of these (between G4 genes *E* and *J* and genes *C* and *D*) are not present in Φ X174. This is shown in Fig. 2. One of the new G4 intergenic spaces is a result of a duplication of the 3' end of the overlapping G4 gene *D* and *E* coding regions which introduces a 32-nucleotide untranslated sequence between the *E* and *J* genes⁵⁵. The other new intergenic space has been introduced by changes in the end of the G4 gene *C* coding region relative to Φ X174, which has resulted in a loss of two gene *C* codons and an insertion of one untranslated A nucleotide between the G4 *C* and *D* genes. In G4 the sequence T-G-A-A-T-G has replaced the overlapping gene *C* and *D* termination and initiation codons (T-G-A-T-G) present in Φ X174 (Fig. 1).

The sequences of the H/A and J/F intergenic regions are about 50% conserved between the G4 and Φ X174 genomes when they are aligned for maximum homology. The large F/G and the small G/H intergenic regions, however, show little sequence homology between the two bacteriophages. Despite the sequence differences, the three large intergenic spaces in both G4 and Φ X174 (J/F, F/G and A/H) can form hairpin loops which are in the same relative position in the sequence and have similar size and geometry. This is illustrated in Fig. 4 which shows the untranslated sequences between G4 and Φ X174 genes *H* and *A*. The similarity of the 3' end of the sequence is a special case because of the presence of a ribosome binding site, a promoter and an mRNA termination site. The conservation of the hairpin loops in the J/F and H/A untranslated regions is believed to be related to the transcriptional termination sites observed in these regions in Φ X174 (see below).

The G4 untranslated region between genes *G* and *F* contain three hairpin loops¹⁷ compared with two in Φ X174 (ref. 31) and one of these in G4 is associated with the origin of G4 complementary DNA strand synthesis. The function of the other hairpin loops in this region in G4 and Φ X174 is not known.

G4 promoters and mRNA termination sites

There is little information on transcription and translation in G4. Three G4 RNA polymerase binding sites have been identified by filter binding experiments³² and these appear to correspond with the three RNA polymerase binding sites observed in analogous experiments in Φ X174 (ref. 33). Two of the Φ X174 RNA polymerase binding sites have been shown by mRNA studies³⁴⁻³⁷ to be the gene *A* and gene *D* promoters, but

Fig. 2 Aligned physical genetic maps of bacteriophage G4 and Φ X174. The G4 and Φ X174 genetic maps are drawn to scale from the G4 (Fig. 1) and Φ X174 (refs 1, 55) nucleotide sequences. The lengths of the untranslated regions are shown above the intergenic spaces. The locations of the three promoters (P_A , P_B and P_D) and the origins of viral DNA strand synthesis (O_V) and complementary DNA strand synthesis (O_C) are described in the text. The position of the nucleotides inserted and deleted in the G4 coding regions relative to Φ X174 have been deduced from an alignment of the complete G4 and Φ X174 nucleotide sequences.

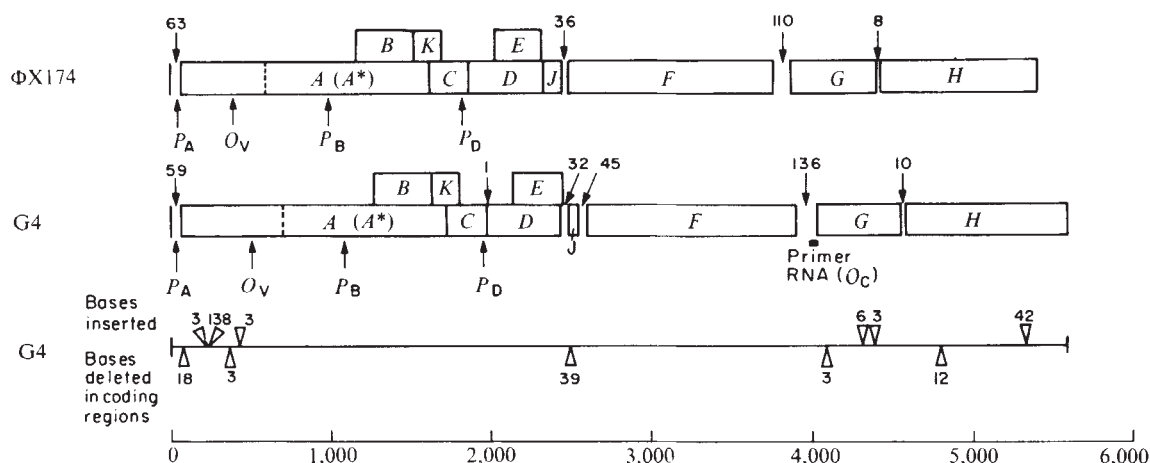


Table 2 Use of codons in G4, Φ X174 and MS2

		G4	Φ X174	MS2			G4	Φ X174	MS2			G4	Φ X174	MS2			G4	Φ X174	MS2
Phe	TTT	33	71	19	Ser	TCT	86	65	15	Tyr	TAT	49	60	9	Cys	TGT	9	15	6
	TTC	61	37	29		TCC	27	19	20		TAC	36	21	32		TGC	9	15	6
Leu	TTA	37	32	17		TCA	40	23	16	Ter	TAA	5	3	1	Ter	TGA	6	8	0
	TTG	20	50	11		TCG	21	25	22		TAG	0	0	2	Trp	TGG	33	31	23
Leu	CTT	53	67	15	Pro	CCT	48	46	17	His	CAT	34	30	6	Arg	CGT	46	56	21
	CTC	44	22	26		CCC	19	11	10		CAC	23	11	9		CGC	47	45	20
	CTA	16	11	15		CCA	21	9	9	Gln	CAA	64	48	17		CGA	12	12	10
	CTG	43	35	9		CCG	16	25	12		CAG	35	62	22		CGG	8	10	11
Ile	ATT	51	75	12	Thr	ACT	59	66	19	Asn	AAT	63	73	17	Ser	AGT	12	16	8
	ATC	57	22	25		ACC	46	27	21		AAC	80	43	28		AGC	6	10	16
	ATA	10	7	19		ACA	38	21	13	Lys	AAA	110	91	19	Arg	AGA	6	16	7
Met	ATG	72	76	20		ACG	19	35	14		AAG	44	56	26		AGG	4	3	6
Val	GTT	51	83	21	Ala	GCT	97	123	26	Asp	GAT	48	79	28	Gly	GGT	63	75	37
	GTC	36	17	21		GCC	39	32	21		GAC	81	62	22		GGC	48	45	16
	GTA	26	15	16		GCA	34	20	21	Glu	GAA	74	36	16		GGA	23	23	12
	GTG	30	20	21		GCG	16	26	23		GAG	31	65	28		GGG	5	5	16

the third binding site is in gene *G* where no mRNA is known to start.

The third Φ X174 promoter—that preceding the *B* gene—has been identified in the Φ X174 nucleotide sequence (ref. 1 and J. Sims and D. Dressler, personal communication). The similarity of the G4 sequence and Φ X174 sequence in the regions of the Φ X174 promoters suggests that the three G4 promoters are in the same location as in Φ X174. The sequences of the Φ X174 gene *A*, *B* and *D* promoters with the analogous G4 sequences are shown in Fig. 3. Note that all six sequences preserve the common feature of having a sequence similar to T-A-T-Pu-A-T-Pu centred about eight nucleotides from the mRNA initiation point^{38,39} and a sequence similar to T-G-T-T-G-A-C-A-A-T-

T-T centred about 35 nucleotides from the mRNA initiation point (refs 40, 41 and J. Majors, unpublished). The 5' end of the major G4 mRNA species can therefore be predicted as A-U-C-A-A (gene *A*), G-U-A-A-C (gene *D*) and A-U-C-G-C-A (gene *B*).

The main mRNA termination site in Φ X174 has been identified at the 3' end of a T-T-T-T-T-A sequence located at the base of the hairpin loop in the untranslated region between the *H* and *A* genes (J. Drouin, unpublished) (Fig. 4). Similar T₆A sequences have been found at the 3' end of RNA transcripts in phage λ (refs 42, 43) and in bacteriophage fd (ref. 44), and in both these cases it has been suggested that the hairpin loop preceding the T₆A sequence is an important signal for transcriptional termination. In G4 there is a T-T-T-T-C sequence instead of a T-T-T-T-T-A at the base of the hairpin loop in the H/A untranslated region (Fig. 4) and from analogy this is most probably the major G4 mRNA termination site.

A minor Φ X174 mRNA termination site has been identified to the 3' side of the hairpin loop in the J/F untranslated region (J. Drouin, unpublished). This hairpin loop is also conserved in G4 but with no sequence homology, and it is not known whether there is a transcriptional termination site in this position in G4

Table 3 Proposed ribosome binding sites in G4

Gene	Nucleotide sequence
A	A-T-C-A-A-A-C- G-G-A-G-G -C-T-T-T-T-C- A-T-G
A*	A-T-C-T-T- G-G-A-G-G -A-G-T-C-A-A-C-T- A-T-G
B	A-G-G-C-T- A-G-G -G-A-A-T-A-A-A-G-A-A- A-T-G
K	C-A-A-G-T-A-C- G-G-A -T-A-T-T-T-C-T-G- A-T-G
C	G-T-G-G-A-C-T-G-C-T-G-G-T- G-G-A -A-A- A-T-G
D	A-C-C-A-C-A- A-A-G-G-A -A-A-C-T-G-A-A- A-T-G
E	C-T-A-T-C- G-A-G-G -C-T-T-G-C-G-T-A-T- A-T-G
J	C-T-T-T- T-A-A-G-G-A-G -T-T-A-T-G-T-A- A-T-G
F	C-T-A-T-T- T-A-A-G-G-A -T-A-C-A-A-A-A- A-T-G
G	A-G-C-C-A-A- A-A-G-G-A -C-T-A-A-C-A-T- A-T-G
H	T-G-A-A-A- T-A-A-G-G-A -T-T-A-T-C-C-T- A-T-G
16S rRNA 3' end	HOA-U-U-C-C-U-C-C-A-C-U-A-G

G4 Ribosome binding sites and possible new overlapping proteins

Table 3 shows the sequences preceding the A-T-G initiation codons of known G4 coding regions. The nucleotides complementary to the 3' end of 16S RNA which are characteristic of ribosome binding sites⁴⁵ are indicated. The five G4 ribosome binding sites that occur in the untranslated intergenic spaces (those for genes *A*, *J*, *F*, *G* and *H*) have different sequences from the same sites in Φ X174 whereas the G4 ribosome binding sites that occur in coding regions of the preceding gene (those for genes *A**, *B*, *K*, *C*, *D* and *E*) are almost identical to their Φ X174 counterpart.

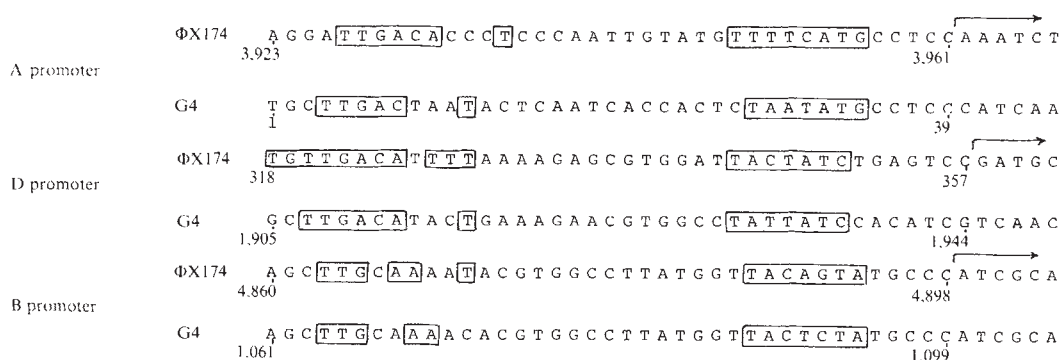
In G4 there appear to be other sites which could code for a protein. There are 13 sites in the genome which have complementarity with the 3' end of 16S ribosomal RNA and are located within 14 nucleotides of an A-T-G initiation codon and followed by an open phase of at least 20 codons (Table 4). Only two of the G4 potential initiation sites are present in Φ X174 (which has others that are not represented in G4 (ref. 46)) and would specify the same length protein. These are the G4 A2a (and A2b in the same phase) and F2 potential proteins (Table 4). It is not known whether any of these sites are used to synthesise a so far undetected protein *in vivo*, but one new G4 overlapping gene, *K*, was predicted in this manner from the nucleotide sequence and the protein was subsequently found³.

G4 has a unique origin of synthesis of its viral and complementary DNA strands which are separated and on almost opposite sides of the genome (Fig. 2). The *in vivo* origin of the G4 viral DNA strand has been located by pulse label experiments^{47,11} and by electron microscope mapping of the 5' end of the tail of late rolling circle replicative intermediates⁴⁸. It is in the *Haemophilus aegyptius* restriction endonuclease fragment *Hae*III2b near the junction of the *H. influenzae* restriction endonuclease fragment *Hind*IIA and D. When the G4 and Φ X174 sequences are compared across this region¹⁷, there are 30 nucleotides (500–529 in Fig. 1) which are identical in both genomes and within this sequence the Φ X174 cistron A protein is known to cleave Φ X174 double-stranded DNA and initiate viral DNA strand synthesis⁴⁹. This cleavage is between the G

Origin of G4 complementary DNA strand synthesis

G4 has single unique origin of synthesis of its complementary DNA strand, both *in vitro*⁹ and *in vivo* (refs 11, 12). Initiation *in vitro* involves synthesis of an RNA primer molecule¹⁰ the sequence of which has now been determined⁵⁰. This 28-nucleotide RNA sequence corresponds to a G4 DNA sequence in the untranslated region between genes *F* and *G* (ref. 17 and J. Sims and D. Dressler, personal communication) and the 5' end of the primer RNA is at nucleotide 3,997 in Fig. 1. The DNA corresponding to the RNA primer sequence can be drawn in the form of a stable hairpin loop (positions 3,972–3,992 in Fig. 1).

FIGURE 3: Promoter Sequences in G4 and ØX174



Two other hairpin loops are formed in the G4 region between genes *F* and *G* and these appear to be analogous to the loops observed in this region in Φ X174 (ref. 31). Φ X174 does not have a loop corresponding to the G4 RNA primer and there is no evidence for a preferred initiation site for complementary DNA strand synthesis in Φ X174.

It is interesting to note that the sequence of 42 nucleotides on the 3' side of the primer RNA loop in G4 (before the 5' start of

[illegible]

Table 4 Possible extra protein initiation sites in G4

Name*	Position†	Sequence‡	Length of protein§
A1	564	C-A-T-T- G-G-A -A-C-C-T-C-C-T-C-T- A-T-G	45
A2a	822	C-G-C-T-T-A- A-A-G-G-A -T-T-T-C-T- A-T-G	78
A2b	900	G-A- A-G-G-T -C-G-C-T-C-G-G-T-G-C- A-T-G	52
F1	2637	G-C- G-G-A -C-C-G-T-G-T-A-C-C-T-C- A-T-G	74
F2	2871	A-T-G- A-A-G-G-A -T-G-G-C-G-T-T-A- A-T-G	38
F3	3285	G-G-C- G-G-T -C-A-T-A-C-A-T-C-C-T- A-T-G	21
G1	3970	C-T-C- G-A-G -T-C-T-C-C-G-A-T-A-C- A-T-G	42
G2	4192	C-A-T-T-C- A-G-G -C-T-T-G-T-G-C-C- A-T-G	28
G3a	4309	C-G-T-T-T-T- G-A-G-G-T -C-G-C-T-G- A-T-G	68
G3b	4444	G-T-C- G-G-T -A-A-T-C-G-C-G-T-T-T- A-T-G	24
H1	4706	A-A-T-G-T-T-G-T- A-G-G-T-G -C-T-A- A-T-G	101
H2	5126	A-C-T- A-A-G-G-A -T-A-C-T-G-T-C-T- A-T-G	78
H3	5444	G-C-T- A-A-G-G-A -C-G-T-G-T-C-C-A- A-T-G	36
16S rRNA		3' _{HO} A-U-U-C-C-U-C-C-A-C-U-A-G 5'	

* Sites are named according to which gene they occur in and the order in which they occur in that gene. Sites labelled a, b, etc. are in the same reading frame.

† Numbers refer to the A of the A-T-G initiation codon.

‡ Only those sequences that have at least three consecutive nucleotides complementary to the boxed sequence at the 3' end of the 16S rRNA are included. Other restrictions are that these complementary sequences must not be closer than two nucleotides and not further than 14 nucleotides from the A-T-G, and must have at least two potential G/C base pairs with the 16S sequence shown boxed. Possible extra initiation sites in the same reading frame as known genes are not included nor are sites containing G-T-G initiation codons.

§ Length of the polypeptide that could be produced starting at the A-T-G initiation codon. Only those sites that could specify a polypeptide of greater than 20 amino acids have been considered.

the primer RNA) has 26 nucleotides in common with phage λ origin of DNA synthesis⁵¹. This common sequence, together with the hairpin loops that are present in both organisms, may represent the dnaG protein (primase) binding site. The equivalent sequence in Φ X174 has only 11 nucleotides in common with G4.

Location of the *Eco*RI and *Eco*B restriction sites in G4

G4 grows only on *Escherichia coli* C strains and is restricted by K12 and B strains and by strains carrying the plasmid RY (ref. 52). The single *Eco*RI restriction enzyme cleavage site (G-A-A-T-T-C) is at position 3,596 (Fig. 1) and the single *Eco*B restriction site (T-G-A-8N-T-G-C-T)^{53,54} is located between positions 4,523 and 4,537 on the complementary strand. Neither of these sites is present in Φ X174.

Evolution of G4 and Φ X174

The comparative G4 and Φ X174 nucleotide sequence presents an opportunity to analyse the mechanism of evolution simultaneously on both the nucleotide and amino acid levels. It is

clear that G4 and Φ X174 are closely related and have the same overall genome structure and organisation of gene function. The main nucleotide differences between the two genomes are most probably explained by single step mutational nucleotide changes and the increased size of the G4 genome (+3.5%) could probably have taken place by duplications of the sort that are still evident at the end of the G4 gene *D* (nucleotides 2,420–2,434 containing the gene *J* ribosome binding site and initiation codon and the gene *D* termination codon are repeated at positions 2,463–2,477). However, any evolutionary description will have to take into account the following observations: (1) the intergenic untranslated regions of G4 and Φ X174 are different in size and sometimes quite different in nucleotide sequence; (2) both insertions and deletions in one genome relative to the other occur in single gene coding regions (genes *A*, *G* and *H*) and occur in units of triplets so that the amino acid phasing is not changed; (3) alternative serine codons (A-G-C to T-C-T) are used in one phage relative to the other in regions of highly conserved amino acids (for example, at positions 1,186 and 1,304 in gene *A*). An A-G-C to T-C-T change cannot take place in single steps without changing the amino acid. These changes may have taken place by the known recombinational

mechanisms acting on the pool of replicating viral DNA or even within a single genome (that is, intramolecular recombination) rather than deletions and insertions on codon unit lengths of DNA.

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letters to nature

Positions and identifications for galactic X-ray sources 2A1822–371 and 2S1254–690

PRECISE positions and optical identifications for the galactic X-ray sources 2A1822–371 (4U1822–37) and 2S1254–690 (4U1254–69), are presented here using the scanning modulation collimator (MC) on HEAO 1 (ref. 1). Briefly, the HEAO 1 MC instrument contains two independent four-grid collimator systems, one having planes of maximum transmission with 30 arc s. FWHM, and the other having 120 arc s FWHM, the overall field of view of each being limited to $4^\circ \times 4^\circ$ FWHM. The planes of transmission of the modulation collimators are inclined at $\pm 10^\circ$ to the scan direction, so that the lines of position from each have an included angle of 20° . Data are folded and binned around the catalogued positions^{2–4} of X-ray sources in the manner described by Gursky *et al.*¹

2A1822–371 ($l^{\text{II}} = 356^\circ$, $b^{\text{II}} = -11^\circ$): data were accumulated in scans across 2A1822–371 for a total of 59 orbits, from 25 to 29 September 1977 UT, when the source was within $\pm 2^\circ$ of the HEAO 1 scan circle. The source was detected at 14.7σ above background in the 30 arc s collimator (MC1) and at 13.5σ in the 2 arc min collimator system (MC2). The strength of the source was measured as 6 UFU (1 Uhuru flux unit = 1.7×10^{-11} erg cm⁻² s⁻¹, 2–6 keV). This is to be compared with maximum catalogued intensities of about 25 UFU^{2,3,6} and a minimum of 6 UFU³. The present observations were thus taken

when the source was weak. Of the multiple possible positions obtained by the instrument, only one falls within either the 2A or 4U error boxes for the X-ray source, this one MC position (of area 0.6 arc min²) being entirely within the 2A box (50 arc min²) and on the edge of the 83 arc min² 4U box. The HEAO MC position for 2A1822–371 is summarised in Table 1.

The first step in identifying the optical counterpart of this source was to carry out multicolour photographic photometry of the above HEAO MC location. The Cerro Tololo Inter-American Observatory 1 m telescope was used on 28–30 April 1978 with the Carnegie Image tube camera. B and R filters were used which combine with the S20 photocathode to give a spectral response closely approaching the Johnson standard colours (the U filter was not used in this case because of scattered moonlight). Enlargements of the B and R plates are shown in Fig. 1, with the HEAO MC X-ray source location overlaid. The star marked S is clearly extremely blue when compared with all neighbouring stars. Subsequent spectrophotometry of star S was carried out 3–4 May UT, using the CTIO 4 m telescope and silicon intensified target (SIT) vidicon, providing digital readout. The very blue nature of the spectrum was readily confirmed, but accurate photometric magnitudes were difficult to ascertain because of poor seeing conditions. However, we estimate magnitudes of $V \sim 16.29$ and $B \sim 16.24$, with errors of 0.25, and a colour index $(B - V) = -0.05 \pm 0.1$. Intense emission lines of the $\lambda\lambda 4640$ – 4650 CIII/NIII blend and $\lambda 4686$ He II were easily visible in ~ 10 -min observations, with measured equivalent widths of 1.9 Å for CIII/NIII and 2.8 Å for He II. A

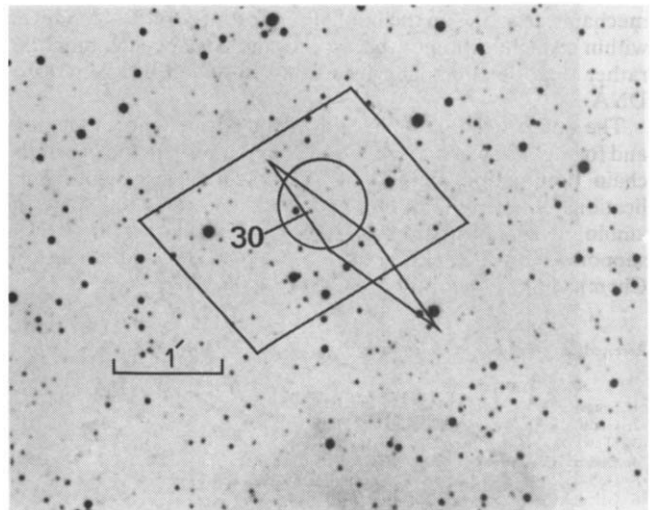
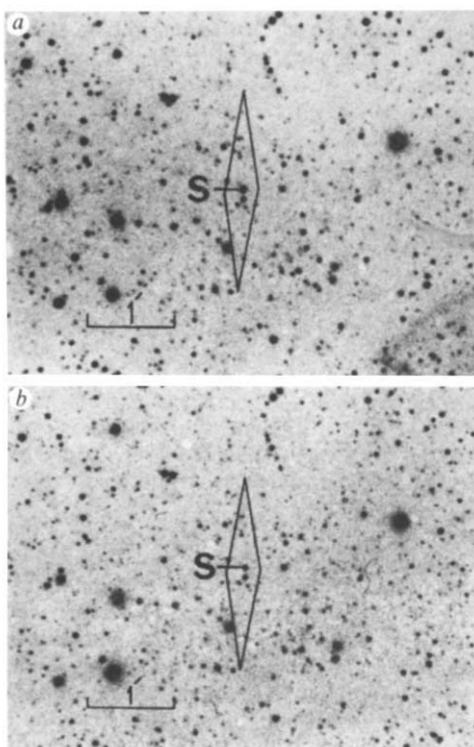
Table 1 HEAO 1 MC positions (1950.0) for 2A1822-371 and 4U1254-69

	2A1822-371		4U1254-69	
	RA	dec	RA	dec
Centre	18h22min22.92s	-37°08'09"	12h54min15.84s	-69°01'20"
90% confidence corners	$\left\{ \begin{array}{l} -2''.3 \\ +12''.6 \\ 2''.6 \\ -12''.1 \end{array} \right.$	-69"	+50"	+49"
		+1"	+8"	-9"
		+70"	-50"	-49"
		-1"	-9"	+9"
Optical counterpart (positional uncertainty 4")	18h22min22.7s	-37°08'3"	12h54min20.9s	-69°01'07"
	star 'S'		star '30'	

systematic uncertainty $\pm 10\%$ in these equivalent widths arises from the estimation of the continuum level. Given the location of this star within the HEAO MC error box, the very blue nature of the spectrum and the presence of emission lines characteristic of X-ray binary systems (see ref. 5), we consider the identification of this X-ray source to be secure.

As the X-ray spectrum of this source has been shown to be relatively hard (energy spectral index $\alpha \sim 0.7$, with hydrogen column density $N_H \sim 8.10^{22}$ atoms cm^{-2}) (ref. 6), a search for X-ray and/or optical pulsations may be worthwhile.

4U1254-69 ($l^{\text{II}} = 303^\circ$, $b^{\text{II}} = -6^\circ$): data from 4U1254-69 (2S1254-690) were accumulated early in the HEAO mission, from 24 orbits between 17 and 19 August 1977 UT. The source was detected at 12σ above background in MC1 and 14σ in MC2, with a measured flux of ~ 6 UFU (2-6 keV), to be compared with previously catalogued values of 23.2 UFU (2-6 keV) (ref. 3) and ~ 25 UFU (2-11 keV) (ref. 4). The source was thus considerably weaker than in previous observations; variability is clearly indicated by a factor of 4, as reported from OSO 7 observations⁶. This galactic source has been accurately

Fig. 1 Finding chart for optical counterpart to 2A1822-371. HEAO MC position (diamond), shown superimposed on our CTIO 1 m B (a) and R (b) plates. Star S is the optical candidate, discussed in the text. North is up and east to the left is both Figs 1 and 2.**Fig. 2** Finding chart for the optical candidate to 4U1254-69, taken from our CTIO 1 m B plate. The HEAO MC position is the diamond, the circle is the SAS 3 position⁴, and the rectangle is the 4U position³. The optical candidate is the star labelled '30'.

positioned by SAS 3 and Fig. 2 shows the overlay of our MC error box (diamond) and the 25 arc s radius SAS 3 circle⁴ on our CTIO 1 m B plate. The coordinates for the MC error box for 4U1254-69 are shown in Table 1.

Using the same equipment described above, U, B and V plates of the 2S1254-690 field showed star 30 of Dower *et al.*⁴ to be relatively blue compared to other stars. Estimated magnitudes from our Vidicon observations are $V = 19.09 \pm 0.1$ and $B = 19.24 \pm 0.1$. Spectra of star 30 were taken on 2 and 3 May 1978 UT, which were summed together (~ 2.7 h integration time) to reveal the $\lambda\lambda 4640-4650$ CIII/NIII blend with an equivalent width of 5.4 ± 1 Å. As star 30 is within both the SAS 3 and HEAO MC error boxes, we again consider this star as a probable optical identification.

Because of the strong UV excesses observed in these two objects, and their relatively high galactic latitude, neither is likely to be substantially reddened. Thus, it is extremely unlikely that they are early type systems (for example, OB supergiants such as Cyg X-1) as this would imply a distance of ~ 100 kpc and corresponding X-ray luminosities $\sim 10^{39}$ erg s^{-1} . Given the low reddening, we may compare their X-ray-to-optical luminosity ratios, L_X/L_{opt} with those of other known X-ray counterparts. Taking the bolometric corrections to be zero for simplicity and consistency, and converting to energy units⁷, we find L_X/L_{opt} to be ~ 600 for 4U1254-69 and ~ 25 for 2A1822-371. Using the same procedure, Sco X-1 has an L_X/L_{opt} of ~ 900 . (The observed colour of 4U1254-69 is very similar to that of Sco X-1; indeed, it may be even more blue.) Thus, 4U1254-69 is remarkably similar to Sco X-1 in this respect, whereas 2A1822-371 is different by more than an order of magnitude. Bolometric corrections are unlikely to account for this effect as the colours of the candidate objects are broadly similar. We suggest that much of the optical luminosity of 2A1822-371 originates in the normal star. The smaller equivalent width of the CIII/NIII blend in 2A1822-371 supports this interpretation.

Assuming the same luminosity for 4U1254-69 as for Sco X-1 ($\sim 4 \times 10^{36}$ erg s^{-1} , 2-10 keV) and a distance to Sco X-1 of 500 pc, we may estimate the distance of 4U1254-69 to be ~ 10 kpc. As far as we can tell from present observations this system has an optical spectrum similar to that of the steady component of the optical-X-ray burster MXB 1735-44 (refs 8, 9), and colours similar to those of the 7.7 s optical-X-ray pulsating source 4U1626-67 (refs 10, 11).

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R. E. GRIFFITHS
H. GURSKY
D. A. SCHWARTZ
J. SCHWARZ

Harvard-Smithsonian Center for Astrophysics,
60 Garden Street,
Cambridge, Massachusetts 02138

H. BRADT
R. E. DOXSEY

Massachusetts Institute of Technology,
Center for Space Research,
Cambridge, Massachusetts 02139

P. A. CHARLES
J. R. THORSTENSEN

University of California,
Space Sciences Laboratory,
Berkeley, California 94720

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Interpretation of tails of X-ray bursts

ALCOCK AND HATCHETT¹ recently proposed that the tails of X-ray bursts^{2,3} observed during burst decay (time scales typically tens of seconds) are due to the small-angle scattering of the original pulse of X rays on interstellar dust grains. To produce the desired tails, and obey constraints set by optical interstellar reddening data, their model uses grains with a typical size of $\sim 3 \mu\text{m}$. Here we give four independent reasons which rule out their model (see also ref. 3).

(1) Bursts from one source in general are similar to one another^{2,4}. However, a burst is sometimes detected with a tail much longer (already pointed out by Alcock and Hatchett) or shorter than is typical for that source. This has been observed for example in bursts from MXB1837+05 (SAS-3, unpublished data) and MXB1728-34 (ref. 5) where the minimum duration of the tails was a few seconds, the maximum ~ 100 s.

Because of the low interstellar reddening of the globular cluster NGC6624, Alcock and Hatchett conclude that the tails of the bursts from MXB1820-30 in this cluster cannot be explained by their model but are intrinsic to the source. Additional evidence for this conclusion is contained in a series of bursts from MXB1820-30, reported by Clark *et al.*⁶. The tails in the bursts became gradually shorter and the last few bursts showed hardly any tails at all. (The tail duration gradually decreased from ~ 25 s to <10 s). Therefore, a large part of the tails in bursts from these sources must be intrinsic to the source.

(2) Different kinds of bursts are emitted by MXB1730-335 (Rapid Burster)⁷. Besides the Type I bursts, the Rapid Burster also emits Type II bursts, whose spectral evolution is very different in that the softening during burst decay is much less pronounced than in the Type I bursts^{7,8}. If the mechanism proposed by Alcock and Hatchett were to explain the tails of the

Type I bursts such softening tails should also be observed after these Type II bursts, but they are not.

(3) Furthermore, in spite of the large distance and interstellar reddening¹⁵ of MXB1730-335 a superposition of 12 small Type II bursts by Ulmer *et al.*¹⁶ shows that these bursts do not have tails longer than ~ 2 s. This observation alone shows that the tails caused by interstellar scattering have a duration ≤ 2 s for the Rapid Burster.

(4) According to Alcock and Hatchett the optical depth τ_x for scattering of X rays on $3\text{-}\mu\text{m}$ grains has to be small. Hence, the energy observed in the tails of X-ray bursts is proportional to τ_x (see their equation (16)). We make the reasonable assumption that the distribution of the large grains follows that of the small ones (which are responsible for the interstellar reddening). Most of the heavy elements in the interstellar medium are probably locked up in grains⁹. Therefore, according to this model, one would expect a positive correlation between the relative strength of the burst tails and the interstellar absorption. The latter is commonly expressed in terms of the equivalent interstellar column density of hydrogen N_H . We tested this prediction by comparing the observed values of N_H , as obtained from fits to the burst spectra⁴ with the ratio ϵ of the energy in the burst tails to the total burst energy. In those cases where optical evidence is available on the interstellar reddening (MXB1659-29; 1735-44 and the galactic centre burst sources)¹⁰⁻¹² good agreement exists with these N_H values, using a relationship between N_H and E_{B-V} (see refs 13 and 14). It is somewhat ambiguous to determine where the tails start, but this is of no consequence; the range of values of N_H is at least a factor of 10, and this is much larger than the uncertainties introduced by the above ambiguity.

There is no correlation between ϵ and N_H for the 10 burst sources studied previously⁴. The values of ϵ for the sources with $N_H \leq 10^{22} \text{ cm}^{-2}$ range from 0.35 to 0.65. For sources with $N_H > 10^{23} \text{ cm}^{-2}$, ϵ ranges from 0.3 to 0.5. These results clearly exclude that the tails are the result of grain scattering, because in that case we would expect values of ϵ to be approximately proportional to N_H .

It may be argued that, as dust particles approximately midway between the burst source and the observer dominate the scattering mechanism, this lack of correlation reveals local concentrations of dust around some of the burst sources. In that case the typical value of the 'non local' column density is $\leq 10^{22} \text{ cm}^{-2}$, corresponding to interstellar extinction values of ≤ 4 mag. According to Alcock and Hatchett this is insufficient to produce a significant observable effect.

The model of Alcock and Hatchett (although it may work at different time scales and lower intensity levels) does not account for the observed tails of X-ray bursts. These tails are predominantly intrinsic to the X-ray burst mechanism.

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JAN VAN PARADIJS
WALTER H. G. LEWIN

Department of Physics and Center for Space Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

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A possible practical application of heavy quark physics

A POSSIBLE method for storing very large amounts of useable energy within small volumes is described here. Our proposal is based on the existence of two new, virtually stable, forms of matter that annihilate each other with enormous energy release, but neither of which annihilates ordinary matter (and can thus be stored easily). The existence of these new forms of matter is suggested^{1,2} by the phenomenology of heavy quarks and also by a large class of widely discussed unified field theories.

It has been proposed^{1,2} that the b quark, the constituent of the T meson³⁻⁵, might be a colour sextet and as such probably be absolutely stable. The possible absolute stability of the b quark has also been explored from a different viewpoint⁶⁻⁸. We assume^{1,2,6} that the electric charge of the b quark equals $-\frac{1}{3}$ times that of the proton. The b quark carries colour and is therefore confined inside colourless hadrons, that is, observable baryons and mesons. The lightest baryons containing a b (or $\bar{b}$) quark are then $B^+ \equiv buu$, $B^0 \equiv bud$, and the lightest mesons are $M^+ \equiv b\bar{u}$ and its antiparticle $M^- \equiv b\bar{d}$. Being each other's antiparticles M^- and M^+ have the same mass: $m_{M^+} = m_{M^-}$, while the B^+ and B^0 masses differ: $m_{B^+} \neq m_{B^0}$. For $m_{B^+} < m_{B^0}$ and $|m_{B^+} - m_{M^+}| < m_p + m_{\pi^+} \approx 1,078$ MeV the hadrons B^+ and M^+ are absolutely stable and B^0 will undergo β -decay. Conversely, for $m_{B^0} < m_{B^+}$ and $|m_{B^0} - m_{M^+}| < m_p \approx 938$ MeV, B^0 and M^+ are absolutely stable. Standard quark model reasoning strongly suggests the validity of these meson-baryon mass-difference inequalities. Thus the meson M^+ and one of the baryons, B^+ or B^0 , are expected to be stable. Whichever baryon is stable (B^+ or B^0) the following three observations apply: (1) neither M^+ nor B^+ , B^0 contain $\bar{u}$ or $\bar{d}$ antiquarks and therefore do not annihilate ordinary matter; (2) the positively charged hadrons M^+ and B^+ can form hydrogenoid atoms, which we denote $\mathcal{M}(M^+e^-)$ and $\mathcal{B}(B^+e^-)$, with ordinary negative electrons. These electrically neutral atoms are stable and, as they contain electrons rather than positrons, they do not annihilate ordinary matter either. Hence they may participate in ordinary chemical reactions; (3) M^+ contains one $\bar{b}$ but B^+ , B^0 each contain one b quark, so that M^+ can annihilate with both B^+ and B^0 .

Several intriguing new possibilities arise. The most attractive one is to assume first stable M^+ and B^+ . The resulting hydrogenoid gases $\mathcal{M}_2$ and $\mathcal{B}_2$ may be stored in conventional containers and transported separately, or the compound $\mathcal{MB}$ may be formed and transported itself. Other more complex chemical compounds would certainly exist. These two new forms of matter $\mathcal{M}$ and $\mathcal{B}$ can be induced to annihilate with considerable release of energy, ~ 10 GeV per reaction, with typical nuclear cross sections.

The case of a stable M^+ and B^0 is more subtle. Perhaps the B^0 can be attached to a conventional nucleus forming an exotic isotope. For example, if a stable heavy deuterium consisting of B^0p^+ exists, then the discussion of the preceding paragraph applies with $\mathcal{B}$ replaced by $B^0p^+e^-$. Other stable or long lived quasi-stable isotopes may exist. If no stable nuclei containing B^0 exist, then this substance would have to be contained by itself, perhaps using a 'magnetic bottle' with a field of non-uniform gradient exploiting the B^0 magnetic moment. In this case there would be no Coulomb barrier to overcome in initiating annihilation reactions.

All these possibilities seem to be within the realm of conventional technology. Stable quarks would, therefore, offer the possibility of storing very high (useable) energies within small volumes. The technological possibilities are self-evident. It is, as we have found, an inexpensive device to store antiprotons.

We emphasise that experimentally both the existence and the stability questions for the B^{0+} and M^+ hadrons are still open. A further problem is to provide sufficient amounts of B^{0+} and M^+ matter in a reasonable time interval. Nevertheless, the prospect of this new energy source is worth pointing out at this time.

Finally, should the b quark not be absolutely stable or sufficiently long lived, heavier quarks may exist which are. If carrying $-1/3$ or $+2/3$ proton electric charges, any such quark would be as good in the applications envisaged here.

Since we submitted this note two experimental searches^{9,10} for stable hadrons in the 4–10 GeV c^{-2} mass range have yielded negative results, thus making the existence of stable b -quark-containing hadrons highly unlikely. One may have to wait for higher quark species. We have also learned of a paper of H. Fritzsch¹¹, in which the results given in refs 1, 2 are independently obtained.

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PETER G. O. FREUND
CHRISTOPHER T. HILL

The Enrico Fermi Institute,
and the Department of Physics,
The University of Chicago,
Chicago, Illinois 60637

Received 3 July; accepted 2 October 1978.

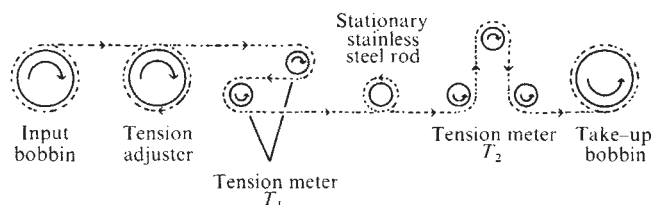
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Dyeing increases the friction of synthetic polymer yarns

WE show here that the friction on metal of poly(ethylene-terephthalate) (PET), nylon-6,6 and polyacrylonitrile (PAN) yarns increases when the yarns are dyed, each with a different dye. This phenomenon is well known in the textile industry, but it has only been investigated quantitatively in one case, the friction of dyed wool on metal¹. Our results show that it is a general phenomenon, and that it is possible to change the mechanical properties of a polymer by the addition of small molecules which have an affinity for the polymer chains.

The PET, nylon-6,6 and PAN yarns were dyed with a disperse, an acid and a basic dye respectively in various conditions (see legends to Figs 2 and 3). The friction of the dyed yarn samples, of the purified but otherwise untreated yarns, and of

Fig. 1 Measurement of yarn friction (see ref. 3 pages 189, 210). Dyed and undyed yarn samples were originally wound on bobbins. The yarn passes round a pulley with a ferromagnetic disk at its centre: the input tension of the yarn is adjusted by moving a permanent magnet nearer or further from the disk. Input tension T_1 is measured by a tension meter consisting of two small pulleys one of which is on a spring loaded arm. The yarn passes once round a stationary steel rod 1 cm diameter then over three small pulleys of a second meter: the central pulley is attached to the plates of a condenser, and its capacitance is recorded continuously and calibrated to measure the output tension, T_2 . The yarn is wound on to a bobbin at a constant speed of 12.8 m min⁻¹. Pulleys and the rod were cleaned with acetone before each determination.



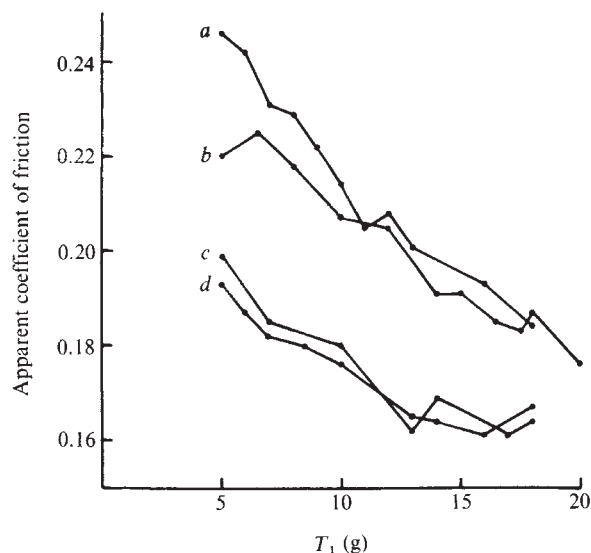


Fig. 2 Decrease in the apparent coefficient of friction of dyed and undyed nylon-6,6 yarn as the input tension is increased: yarn dyed with *a*, 10% and *b*, 7% dye in the dyebath, *c*, undyed yarn, *d*, yarn treated in the same conditions as the dyed yarns but with dye omitted from the bath. Samples of nylon-6,6 yarn (100 m) were purified by soxhlet extraction for 12 h with 60/40 v/v benzene-methanol mixture. The ratio of the dyebath volume to dry weight of the yarn was 30:1. Each yarn sample was immersed in distilled water containing 4% v/wt formic acid for 15 min at 50 °C (all percentages are based on the weight of the yarn). 1% Nylomine Black A-B (ICI), an acid dye, was dissolved in a small volume of the dilute formic acid solution and added to the dyebath, which was raised to 95 °C in 45 min with stirring, and maintained at 95 °C for a further 45 min. The bath containing the yarn was allowed to cool to room temperature. The yarn was rinsed in distilled water and dried at room temperature. Further samples of nylon-6,6 yarn were dyed in the same way in baths containing 2–10% dye (increments of 1%). In the control (*d*), a yarn sample was treated in the same way, except that dye was omitted from the bath. Weight of the yarn, 0.252 g m⁻¹; delustrated with 0.38% TiO₂.

the yarns treated in the same conditions as the dyed yarns but with the dye omitted from the bath (blank dyebath), were measured using a capstan apparatus (Fig. 1) in which the yarn passes round a stationary stainless steel rod at a constant speed. The apparent coefficient of yarn friction on metal is calculated from the yarn tension T_1 before it passes round the rod and the tension T_2 after the rod.

Assuming that the coefficient of friction of the yarn, μ is related to the frictional force F and the normal force W by:

$$\mu = \frac{F}{W} \quad (1)$$

and assuming that the yarn is inextensible and completely flexible, it can be shown (page 374 ref. 2; page 47 ref. 3) that in the capstan experiment

$$\mu = \frac{1}{2\pi} \ln\left(\frac{T_2}{T_1}\right) \quad (2)$$

It has been shown experimentally that μ determined in this way for a given yarn is not a constant but varies as the input tension T_1 is varied (page 47 ref. 3). μ also depends on the diameter of the rod³ and on the speed of the yarn⁴. This is because equation (1) is not strictly obeyed by polymers sliding on metal, and because yarns are not completely flexible and inextensible^{5,6}. The rod used and the speed of the yarn were the same throughout our experiments, and Fig. 2 shows how the apparent coefficient of friction of undyed and dyed nylon-6,6 yarn, calculated from equation (2) alters as T_1 is varied. Dyed and undyed PET and PAN yarns show a similar decrease in the value of μ as T_1 is increased.

The friction of each yarn purified but undyed, treated in a blank dyebath, and treated with various amounts of dye, was compared by calculating the apparent values of μ at two chosen values of T_1 . The results (Fig. 3) show that for nylon-6,6, PET and PAN yarns the yarn friction on metal increases as the amount of the appropriate dye in the dyebath is increased (that is, as the amount of dye absorbed by the yarn is increased). The comparison of dyed yarn with two controls (undyed and blank dyebath) suggests that the greater friction of the dyed yarns must be due to the dye absorbed by the yarns and cannot be

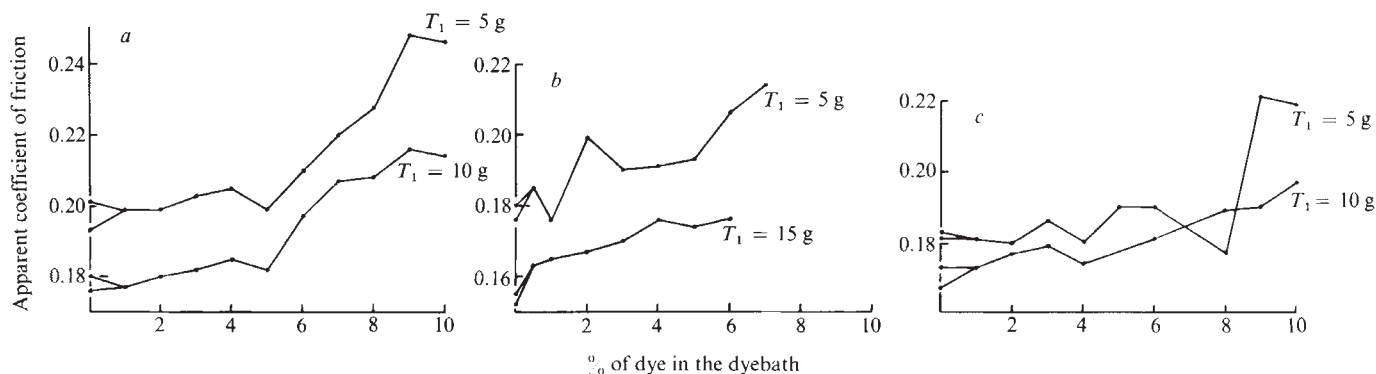


Fig. 3 Increase in the apparent coefficient of friction of nylon-6,6 (*a*), PET (*b*) and PAN (*c*) yarns as the weight of dye in the dyebath is increased. Determinations were made at 5 g and 10 g or 15 g input tension. 100 m samples of all three yarns were purified, dried and weighed as for Fig. 2. Nylon-6,6 yarn was dyed as described for Fig. 2. The ratio of the dyebath volume to the yarn weight was 30:1. An aqueous 0.3% solution (v/v based on the volume of water) of Optinol B (Yorkshire Chemical Co.) was adjusted to pH 5 with acetic acid and heated to 60 °C. A PET yarn sample was placed in it. After 10 min, 0.5% Serilene blue 2G (Yorkshire Chemical) was dissolved in a small volume of the Optinol B solution and added. After stirring for 5 min at 60 °C the temperature was raised slowly to boiling and maintained at the boil for 1 h. The dyebath containing the yarn was allowed to cool to room temperature and the yarn was rinsed in distilled water and dried in air. Further yarn samples were dyed in the same way in dyebaths containing 1–7% dye. Another yarn sample was treated in the same way in a pH 5 Optinol B solution without dye. Weight of the PET yarn, 0.044 g m⁻¹; delustrated with 0.26% TiO₂. A sample of PAN yarn was wetted with distilled water and put into a dyebath containing 1% acetic acid, 1% sodium acetate, 1% Synacril blue R (ICI) and 1.9% Ataxal LC-RA (ICI) cationic retarder, at 95 °C. The dyebath was raised to the boiling point over 10 min. Boiling was continued for 45 min, and then the dyebath containing the yarn was cooled to room temperature and the yarn was removed and rinsed in distilled water. The procedure was repeated using 2% dye and 1.6% retarder, 3% dye and 1.3% retarder, 4% dye and 1.0% retarder, 5% dye and 0.8% retarder, 6% dye and 0.5% retarder, and 7%, 8%, 9% and 10% dye without retarder. A sample of yarn was also treated at the same temperatures for the same times in a bath containing only 1% acetic acid, 1% sodium acetate and 1.9% retarder. Weight of the PAN yarn, 0.124 g m⁻¹; delustrated with 0.32% TiO₂. For the nylon-6,6 and the PAN yarns the higher value of the apparent coefficient of friction at 0% dye was the value obtained with the purified yarn and the lower value was the value obtained with the yarn treated in a blank dyebath. For the PET yarn the opposite was found.

explained, for example, by the removal of lubricant from the yarns during dyeing, or by deposition of oligomers on the surface of the PET yarn during dyeing.

In the simple adhesion theory of friction, Bowden and Tabor (ref. 2 pages 52, 214) propose that when one material is sliding over another the coefficient of friction μ is equal to s/p where s is the force per unit area necessary to shear the softer material, and p is the pressure at which the softer material ceases to deform elastically and begins to flow plastically (that is, p is the yield pressure of the softer material). Our results imply that in drawn synthetic fibres, dyeing increases the shear strength of the fibres in the direction of the fibre axis more than it increases the yield pressure. This may be a consequence of the preferred orientation of the polymer molecules in the fibre direction. Small molecules, not necessarily coloured, with an affinity for the polymer chains may alter the mechanical properties of a polymer by forming weak, non-covalent crosslinks between the chains.

K. HEPWORTH
A. HEWA-KAPUGE
D. L. MUNDEN
P. T. SPEAKMAN

Department of Textile Industries,
The University,
Leeds, UK

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Horizontal structure of scintillation-producing irregularities in southern mid-latitudes

THERE is little information on the instantaneous macroscopic structure of scintillation regions affecting radio communications from satellites. The horizontal structure of scintillation regions has been studied using one or two orbiting satellites or a geostationary satellite. In these studies the temporal and/or spatial resolutions of the methods were poor which made it difficult to evaluate the latitudinal and longitudinal distributions of scintillations over a short period. The temporal resolution is important as scintillation patches seem to drift with a median velocity of 100 m s^{-1} , changing their position from one satellite pass to another¹. To overcome these difficulties I have used all the available VHF transmissions (frequency range: 136-150 MHz) from the following non-synchronous satellites (satellite orbit's inclination is given in brackets): six US Navy Navigational Satellites (90°), NOAA 3 and NOAA 4 (102°), Nimbus 4 (98°), Nimbus 5 and Nimbus 6 (100°), Copernicus (35°), Geos 3 (102°) and Kiku (47°). Amplitude transmissions from these satellites (positioned at altitudes: 700-1,500 km) were recorded on a continuous basis during the periods of increased scintillation activity: summer (November-January 1976-77 and 1977-78) and winter (May-July 1977). The recording station was situated at Brisbane (27.5° S and 152.9° E geogr. lat and long, and 37.2° S invar. lat). The definition of scintillation index (SI) has been given elsewhere².

On occasions scintillation activity coincided with a relatively large number of satellite passes during a period of one hour or less. It was assumed that the position of scintillation patches did

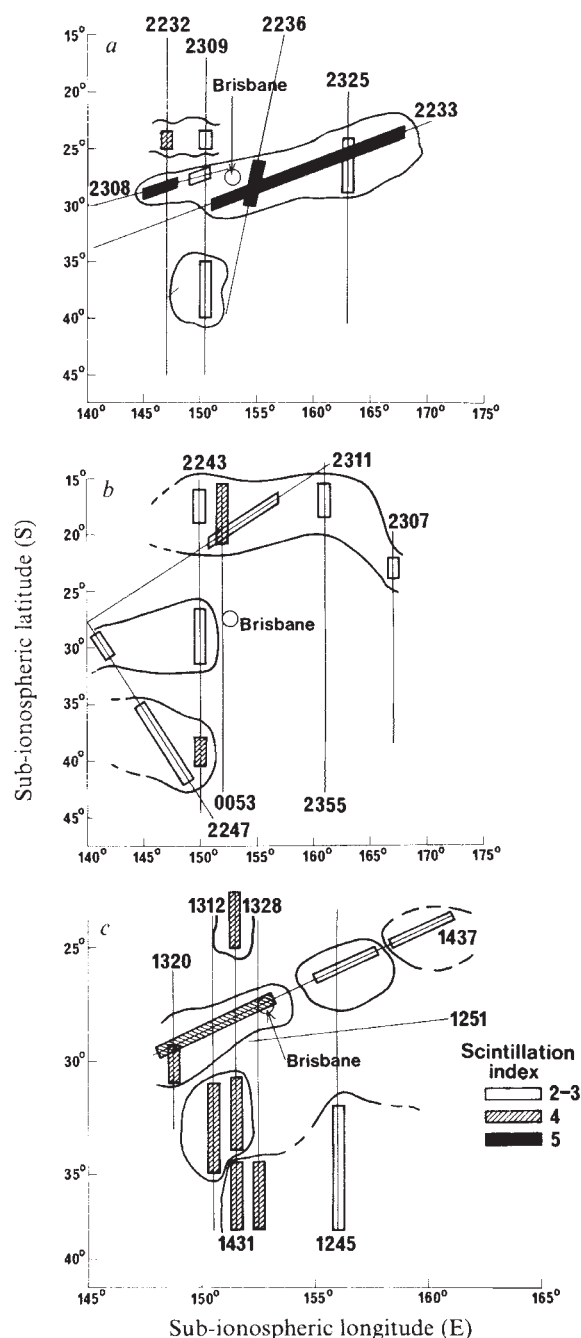


Fig. 1 Examples of sub-ionospheric scintillation regions (rectangular and curves) derived from amplitude scintillations in the VHF transmissions from orbiting satellites. Thin lines refer to the satellite orbits in the vicinity of the recording station at Brisbane. The numbers indicate the local times (150° EST) of the nearest predicted approaches of satellites. The magnitude of scintillation index (SI) is indicated whenever scintillations are present. *a*, 19 January 1978; *h* = 400 km; *b*, 3-4 January 1977, *h* = 400 km; *c*, 3 July 1977; *h* = 100 km.

not change greatly from the first to the last recorded pass during this time. Figure 1 shows examples of horizontal extents of scintillation patches recorded for closely spaced satellite passes during summer night-time (*a* and *b*) and winter daytime (*c*).

Inspection of data from a vertical-incidence ionosonde operating at the same site indicated the presence of intense spread-F and sporadic-E activity coinciding with the periods (*a*, *b*) and (*c*), respectively. From this it was assumed that scintillation-producing irregularities were positioned at altitudes of 400 km (night) and 100 km (day). In Fig. 1*a* scintillations in the

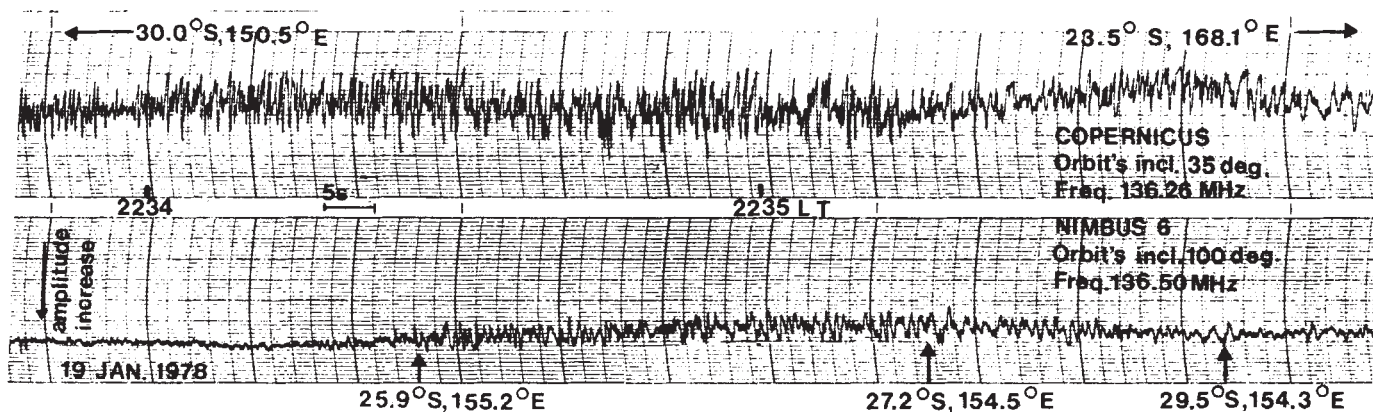


Fig. 2 An example of two simultaneous amplitude recordings of radio-satellite transmissions at frequencies of 136.26 MHz and 136.50 MHz from two orbiting satellites moving mainly in longitude (upper trace) and latitude (lower trace). The sub-ionosphere positions of the limits of scintillation regions are indicated by arrows; the central arrow at 27.2° S and 154.5° E refers to the same sub-ionospheric position for both satellites.

vicinity of Brisbane consisted of an elongated patch extending from about 145° to 170° E longitude. The latitudinal extent was narrower as can be seen from the amplitude data for the north-south passes at 22.36 and 23.25 LT. In particular, two almost simultaneous passes at 22.36 LT (north-south) and 22.33 LT (east-west) revealed a ridge-like formation in scintillations. This can be further seen in Fig. 2 which shows the amplitude scintillation data for these two passes. It is evident that scintillation activity decreased rather rapidly for the north-south pass (Nimbus 6) and continued unbroken for the east-west pass (Copernicus). In addition to the main scintillation ridge two scintillation patches were recorded north and south of the station. The presence of longitudinally extended scintillations is also evident in the second period (Fig. 1b) when a sheet-like irregularity was formed north of the station. Two isolated irregular patches were formed west and south-west of the station. Several isolated patches were recorded during daytime in winter (Fig. 1c). Once again scintillation causing irregularities had a large longitudinal extent. It is interesting that the irregularity positioned between 150° and 152° E was detected by the east-west pass (14.37 LT) only; no scintillations were recorded in this region for north-south passes at 14.31, 13.28 and 13.12 LT. The phenomenon was not isolated and could also be seen in Fig. 1a when scintillation patches recorded for east-west pass at 23.08 LT were not present for two north-south passes at 22.32 and 23.09 LT.

It was possible to obtain 12 records as in Fig. 1 for which at least three satellite passes were recorded with an average time separation of 20 min. In all cases minimum longitudinal extent of scintillations varied from 20° to 30°. Latitudinal extents varied from 3° to 12°, the median value being from 4° to 6°. The horizontal extents of scintillation patches recorded for low orbit's inclination satellites (east-west passes) were several times larger than those for polar orbit's satellites (north-south passes).

Similar results for scintillation occurrences in southern mid-latitudes were also reported from a single polar orbit's satellite transmitting at 20 MHz (ref. 3). It was found that night-time scintillation-producing irregularities occurred in patches with the horizontal extents of 2° to 3° in latitude and more than 15° in longitude. The presence of longitudinally extended irregularity sheets was also reported from a satellite scintillation experiment in Alaska⁴. The ridge-like enhancement in scintillations was associated with the occurrence of the auroral to subauroral transition boundary in the ionospheric total electron content. My results indicate, however, that the sheet-like irregular regions are not limited to a particular spatial position but can occur at various latitudes near the recording station. This might point to a hitherto unrecognised structure of irregularities characterised by a preferential longitudinal extent. A possible

link can be established between this type of irregularities and formation of mid-latitude stable auroral red arcs (SAR) which are aligned in longitude with constant L-shell. Their latitudinal extent is limited between 2° and 4° (ref. 5). There is evidence that SAR are associated with ionospheric scintillations in the UHF range of radio-satellite transmissions⁶.

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L. A. HAJKOWICZ

Department of Physics,
University of Queensland,
St Lucia, Australia 4067

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Detection of ^{14}C using a small van de Graaff accelerator

THERE have been several recent reports of the detection of ^{14}C in modern carbon samples using large van de Graaff accelerators¹⁻³ and cyclotrons⁴ as mass spectrometers. The attraction of using such systems is the possibility that very small samples (~5 mg) might be quickly and accurately dated. We have considered the design criteria for a mass spectrometer incorporating a

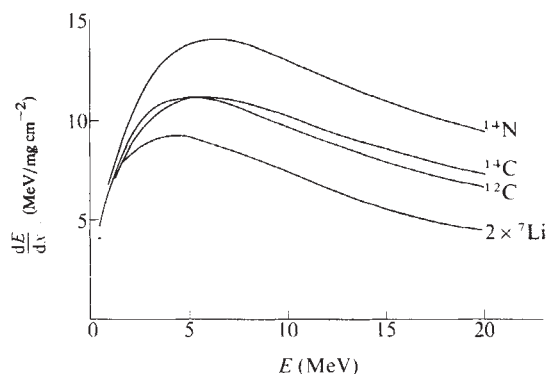


Fig. 1 Energy loss of various ions in the counter gas.

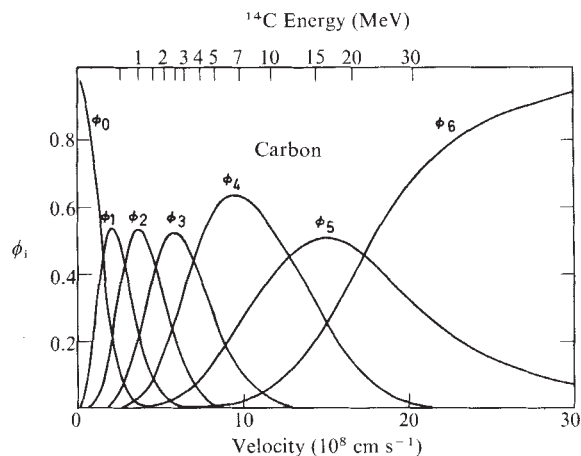


Fig. 2. Charge state fractions for ^{14}C ions after passing through thin carbon foils.

tandem van de Graaff accelerator to be built specifically for carbon dating and in this letter we present data which show that the measurements are possible using much smaller and simpler systems than those discussed previously.

The most important system parameter to establish is the required terminal voltage as this will set the size and cost of the facility. The chief advantage of using a high energy mass spectrometer is that ions can be uniquely identified by measuring their energy and specific energy loss. The energy loss depends on the energy, mass and charge of the nucleus as shown in Fig. 1 (ref. 4). It can be seen from Fig. 4 that at low energy this identification becomes extremely difficult. In practice a clean measurement can be made for carbon ions if they have an energy of more than 5 MeV after passing through the dE/dx part of a gridded ionisation detector⁶. If the counter is set to give two dE/dx measurements with about 1 MeV loss in each, and if there is about 1 MeV loss in the counter window and in the poor field region near the window, then the minimum safe energy is 8 MeV. Figure 2 (ref. 6) shows the charge state fractions for ^{14}C ions being stripped in thin carbon foils. It can be seen that at 2.5 MeV the stripping to the 3^+ charge state reaches a maximum

so that a satisfactory design would have the tandem terminal at 2.5 MV producing 10 MeV $^{14}\text{C}^{3+}$ ions.

Furthermore recent measurements at Rochester have shown that doubly charged metastable molecular ions of mass 14 exist whose lifetimes are long enough for them to introduce a considerable background. This problem can be avoided by selecting the 3^+ charge state, for which the lifetimes of metastable ions are known to be very short.

We have demonstrated that 2.5 MeV is a suitable choice of operating voltage using the experimental system shown in Fig. 3.

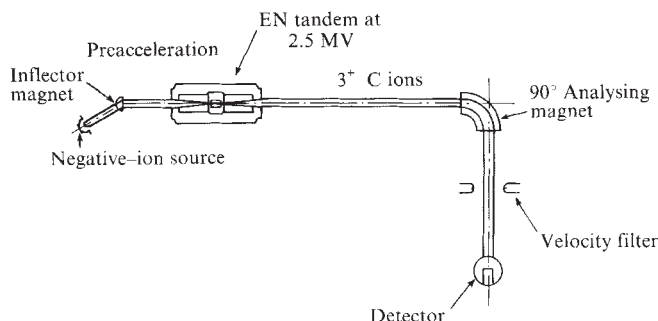


Fig. 3. Beam transport system for the ^{14}C mass spectrometer.

The carbon samples were introduced into a reflected beam sputter source⁸ in the form of graphite pellets made by pyrolysing methane at a pressure of about 500 torr. In this type of ion source a beam of caesium ions, produced by surface ionisation, is accelerated and then focused on to a pellet from which some of the sputtered particles emerge with a negative charge. The intensity of our $^{12}\text{C}^-$ beam was of the order of $5\ \mu\text{A}$ and the intensity of the mass 14 beam was found to be about 3 nA, consisting mainly of $^{12}\text{CH}_2^-$ and $^{13}\text{CH}^-$ and a small fraction of $^{14}\text{C}^-$. For a modern carbon sample, this fraction corresponds to about $37\ ^{14}\text{C}^{3+}$ ions per second arriving at the detector. After acceleration and stripping the beam was momentum analysed to select the 3^+ , mass 14 beam with a resolution of $1:10^3$. A Wien filter with a velocity resolution of 5% removed most of the ions which had the correct magnetic rigidity but which suffered charge exchange outside the gas stripper. The resulting ions were detected in a gridded ion chamber filled with isobutane at

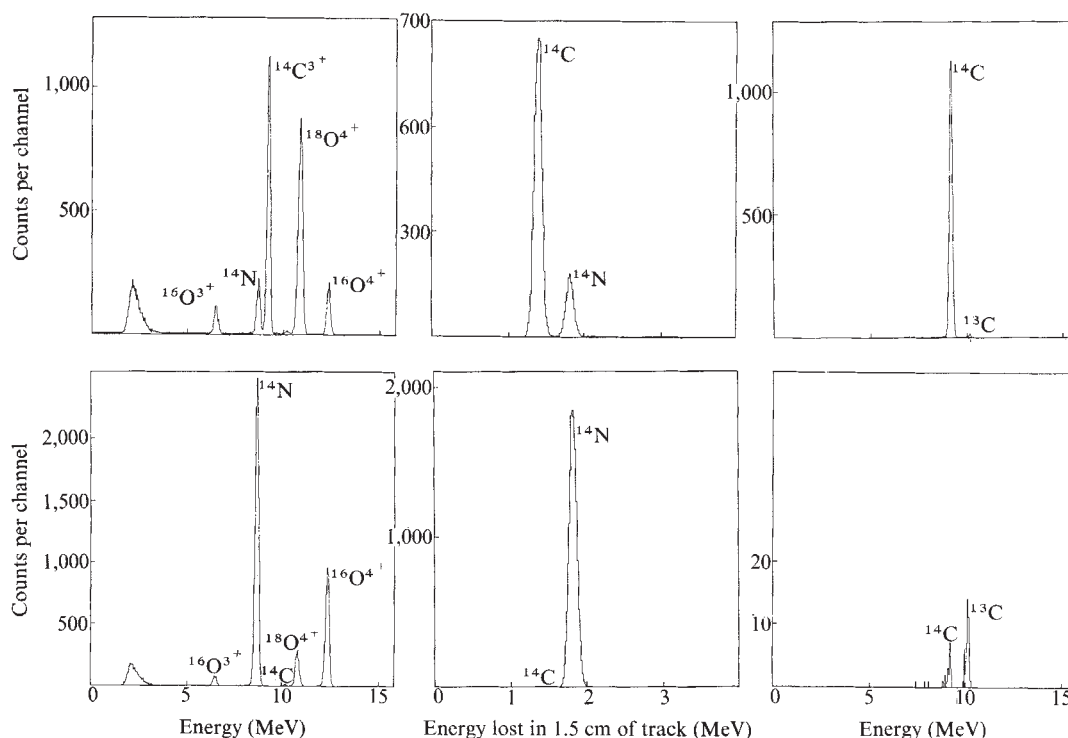


Fig. 4 Energy and energy loss spectra obtained for modern (top line) and old (bottom line) carbon samples. Left, total energy spectrum for carbon; centre, ΔE_2 for $^{14}\text{N} + ^{14}\text{C}$; right, total energy spectrum for carbon ions.

20 torr. The anode of this chamber is split into three sections running normal to the ion trajectories so that two measurements of energy loss (dE/dx) could be made in addition to the total energy. For each ion entering the detector, the ionisation measurements were digitised and recorded on magnetic tape using an on-line computer. The data were then processed to produce various spectra which allowed the ^{14}C count to be determined. Further experimental details may be found in ref. 9.

Typical data for modern and old carbon samples are shown in Fig. 4. It is clear from the energy spectra that the energy resolution obtained (150 keV FWHM) is sufficient to separate the carbon isotopes cleanly. The dE/dx resolution is sufficient to separate the ^{14}N group from the ^{14}C . Interestingly, the nitrogen group is quite intense at these energies. It was established by scanning the terminal voltage that this nitrogen comes from injected NH^- ions which charge exchange somewhere in the accelerator tubes. By observing that there was no shift in the dE/dx peak for the ^{14}C groups from old and new samples we could rule out a significant contribution from $^{13}\text{CH}_3^+$ or $^{12}\text{CH}_2^+$ molecules in this measurement. The background from misidentified particles was less than one count per day.

Our measurements indicate that errors due to cross contamination of samples in the ion source are less than 0.1%. Improved ion sources can produce over 100 μA of $^{12}\text{C}^-$ ions, so that 0.1% counting statistics could be achieved in a few hours. In order to make abundance measurements to this accuracy, however, it will be necessary to monitor closely the source output and the transmission through the accelerator. We propose to achieve this by pulsing the injection system to accelerate the ^{12}C and ^{13}C beams for a few microseconds every millisecond. These beams would be measured in separate ports on the analysing magnet. In addition the ion source will alternate between the unknown sample and a standard. It should then be possible to date accurately small archaeological or environmental samples or the products of laser enrichment as discussed in the previous article.

G. DOUCAS
E. F. GARMAN
H. R. MCK. HYDER
D. SINCLAIR

Nuclear Physics Laboratory, Oxford

and

R. E. M. HEDGES
N. R. WHITE

*Research Laboratory for Archaeology,
Oxford University,
6 Keble Road, Oxford, UK*

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Enrichment of ^{14}C and radiocarbon dating

THE very low natural abundance of ^{14}C (10^{-12} times that of ^{12}C) and its exponential decrease in time require exceptionally sensitive methods for its measurement in the dating of archaeological and environmental samples. Present methods count β emission from the nuclear disintegration and so require substantial samples (1–5 g), long count times (1–3 d), and great attention to

ensure a low level background. Nevertheless, they have been developed to a point where a reproducibility of 0.025% (one standard deviation) has been attained.¹ Here we describe experiments in which dilute ($^{14}\text{C} = 10^{-4} \times ^{12}\text{C}$) samples for CH_2O were enriched by a factor up to 100 by selective photolysis of an individual rotational line in the UV spectrum with a tunable laser. Full details of the experiment will be published elsewhere². This letter summarises the process and develops its relationship to existing and potential carbon dating methods.

Formaldehyde is a convenient molecule for isotopically selective photolysis because it has a well-resolved spectrum in the near UV with a quantum yield for dissociation of near unity. From 350 to 340 nm dissociation is entirely due to $\text{CO} + \text{H}_2$, whereas from 330 to 290 nm about 30% of the quantum yield is to $\text{H} + \text{HCO}$ (ref. 3). If the radicals are not scavenged the enrichment can be reduced. Enrichment takes place when the molecule is photolysed at a frequency where the $^{14}\text{CH}_2\text{O}$ absorption cross section is much greater than that for the other isotopes. Previous work⁴ has shown that a tunable laser with modest bandwidth (~ 20 GHz) could enrich ^{12}C from a mixture of ^{12}C and ^{13}C formaldehyde, and that enrichments of up to 30 could be achieved for some C and O isotopes using ion lasers⁵. Because of the vibrational band envelope structure of the electronic transition in CH_2O it is to be expected that enrichment of a heavier isotope from a mixture will be more difficult, and a necessary preliminary to the enrichment described here was a high resolution survey of the absorption spectra of $^{14}\text{CH}_2\text{O}$ and $^{12}\text{CH}_2\text{O}$ throughout most of the spectral region of 290–350 nm.

Absorption spectra were obtained with a tunable, frequency doubled dye laser (either a Hänsch type N_2 pumped laser, or the Chromatix CMX-4) transmitted in two passes through 2–3 m of 33% $^{14}\text{CH}_2\text{O}$ at about 3 torr, and through 6 m of CH_2O at 10 torr. The laser frequency bandwidth was around 3 GHz (0.1 cm^{-1}). More than 30 $^{14}\text{CH}_2\text{O}$ lines were noted in which the absorption cross section ratio was measured to be greater than 50, and also where the $^{14}\text{CH}_2\text{O}$ absorption cross section itself was greater than $5 \times 10^{-20} \text{ cm}^2$. Figure 1 compares high resolution spectra of $^{12}\text{CH}_2\text{O}$ and $^{14}\text{CH}_2\text{O}$ where such an enrichment line is to be found (at 331.41 nm). The absorption ratio at the spectral resolution used here is 300 ± 100 . The majority of these enrichment lines were in the region 318–340 nm despite the fact that at longer wavelengths the isotope shift is less.

Enrichment was carried out by photolysing a sample of CH_2O containing 0.01% $^{14}\text{CH}_2\text{O}$ in a 3-m cell with two passes for 1–2 h: laser power was typically 0.2–0.5 mW. The CO so produced, in micromole quantities, was collected by trapping with liquid helium, estimated by gas chromatography, and measured for radioactivity with a gas conductivity meter. The specific activity was compared with that obtained from the CO produced by photolysing an equivalent sample of CH_2O with broad band light (mercury lamp), and the ratio of the two specific activities gave the enrichment factor. Enrichment was performed at 318.59 nm and 326.99 nm. The latter wavelength corresponded to the best projected enrichment value and enrichment in the region of 100 was achieved without any attempt at radical scavenging. Improvements to this figure can be expected through minimising pressure broadening effects and improving the laser bandwidth and frequency stability.

As ^{13}C constitutes 1% of natural C, it will become the predominant isotope at enrichments (with respect to ^{12}C) of over 100. Therefore to increase the proportion of ^{14}C in a sample beyond a factor of 100, enrichment with respect to ^{13}C must also be achieved. While frequencies for the optimum enrichment against one isotope will not coincide with those for the other, a study of the overlap of ^{12}C -, ^{13}C - and ^{14}C - H_2O spectral lines shows that substantial enrichment (>10) against ^{13}C can occur simultaneously with high enrichment (≥ 100) against ^{12}C .

We now assess the relevance of laser enrichment to ^{14}C age determination. Enrichment by thermal diffusion of massive samples is already carried out at several radiocarbon laboratories; its requirements of very large samples (~ 40 g), long

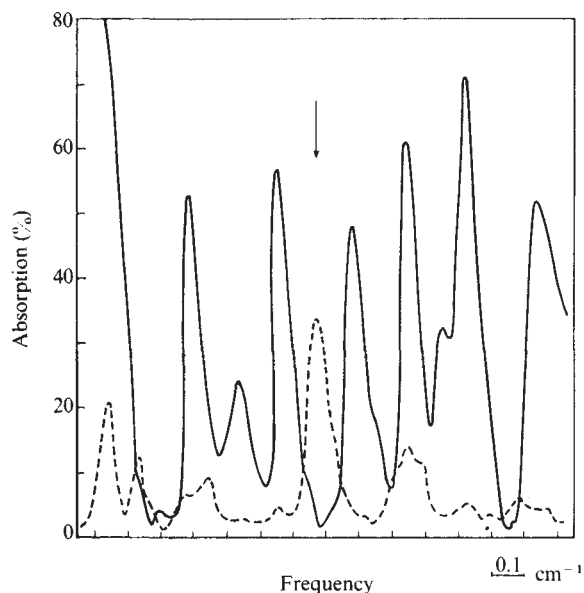


Fig. 1 Comparison of high resolution spectra of $^{12}\text{CH}_2\text{O}$ and $^{14}\text{CH}_2\text{O}$. $^{14}\text{CH}_2\text{O}$ absorption has been reduced by 30 to fit on the same scale.

times (3 weeks) and low enrichment for sufficient product (factor of four) restrict its application to samples too old to date by standard methods ($>40,000$ yr, $<70,000$ yr).

First we consider the practical implications for a laser enrichment system; formaldehyde is relatively easy to prepare, either by the catalytic reduction of CO_2 to methanol, followed by catalytic oxidation, or by the mercury photosensitised reaction of H_2 with CO (ref. 5). Its propensity to polymerise requires that the monomer be stored at 77 K, or at low pressure (<20 torr) in apparatus free from adsorbed water. The photolysis product, CO , is easily extracted in distilled form from the photolysis cell.

The major limitation is the laser energy required to enrich macroscopic amounts of sample. A $^{14}\text{CH}_2\text{O}$ absorption cross section of 10^{-19} cm^2 implies that a photon flux of 0.7 mW cm^{-2} will convert 20% of the $^{14}\text{CH}_2\text{O}$ per hour. This was confirmed in our photolysis experiments. In a carefully designed system it is clear from the effect of the laser linewidth on the measured isotopic cross section ratio that a bandwidth of less than the Doppler width (of 2 GHz) is desirable. To photolyse 50% of the ^{14}C in a 1 g sample, a laser energy in the region of 100 mW hr is necessary. In general the process is likely to be most useful when extracting 20–50% of the ^{14}C , as the enrichment factor starts to fall once the ^{14}C is significantly depleted. In practice we may expect the required energy to be higher by perhaps a factor of two. Where accurate knowledge of the enrichment factor is needed (for example, in the dating of 'young' samples), simultaneous photolysis of a calibrated standard in a separate cell can be carried out. In this way variations in laser frequency and bandwidth are compensated and do not cause systematic changes in the enrichment factor. The design of the laser will depend on the power expected. It is unfortunate that, despite a careful search, no lines for enriching ^{14}C were found to coincide with the frequencies available from high powered rare gas ion lasers. While it is possible that new lines will be available, the chance of coinciding with an enrichment line is very small. Possible tunable laser systems include Raman shifted tunable rare-gas halide lasers, or dye lasers either frequency doubled following pumping by a high repetition rate Cu vapour laser or a high power cw ion laser, or the direct lasing at 330 nm of *p*-terphenyl, pumped by a KrF laser.

There are three possible approaches to ^{14}C abundance measurements—counting ^{14}C atoms or ions after mass filtering, counting the nuclear disintegrations and measuring the half life, or integrating the radioactivity over a period of time. Only the

second method has been used so far for the routine production of dates.

As 1 g of modern carbon has a disintegration rate of 15 d.p.m. counting methods require samples in the 1–5 g range to be counted for 1–2 d for adequate statistics. The advantage of enriched samples is in permitting much smaller counters to be used, thus reducing the background level (which increases with the counter volume). For a modern sample a minimum of 0.5 g is required to accumulate 1% statistics in 24 hours (with negligible background), and laser enrichment could offer little advantage. (The slight gain in accuracy by reducing the background would be offset by inaccuracies in estimating the enrichment factor). But there is a gain for older samples, provided sufficient material can be processed. Consider the enrichment of a 5 g sample of 38,000 yr age. If 25% of the ^{14}C is extracted, and the typical background of 1 c.p.m. is reduced to 5 counts per hour, in 24 hours of counting the background is estimated to 10% statistics, while the signal is estimated to 4%, and the overall statistical error is about 8%, or 1% of the date. The processing of 5 g samples is rather beyond present laser power capabilities, but it would not be unreasonable to expect these to increase by a factor of five in the next few years.

Integrating the radioactivity, for example in a photographic emulsion or thermoluminescent dosimeter, frees the sample from expensive counting apparatus and allows integration for a long period. In natural samples the dose rate is comparable to the cosmic ray and residual dose backgrounds, but 100-fold enrichment would reduce these to negligible levels⁶. The sample size needed can be very small, and so is much better suited to currently available lasers. For example, 10 mg of carbon enriched to 0.1 mg (from a 40 mg sample) gives a 1 mrad dose in three weeks to a conveniently small quantity of phosphor (Dy:CaSO_4), and while thermoluminescent measurements are unlikely to have the accuracy of conventional counting the advantages of this approach in economy and speed are worth pursuing. In principle the same advantages apply to track counting in emulsions⁷, where the level of background tracks can be made negligible and therefore do not require sophisticated discrimination.

If all the ^{14}C atoms are detected, 1 mg of modern carbon contains about 4×10^7 atoms. While the degree of mass filtering from ^{12}C and ^{13}C is enormous, mass spectrometric systems could have total efficiencies in the region of 5–0.1% so that a requirement for modern carbon of 1 mg samples is reasonable. Such accuracy should make it possible to date many new materials, such as CO_2 in glacier cores, foraminifera in ocean cores, carbon in archaeological iron and single seeds. The first serious attempt⁸ to detect ^{14}C mass spectrometrically was made with a two-stage mass spectrometer of $^{14}\text{C}^{15}\text{N}^-$. This failed because of high backgrounds from molecular impurities at this mass. The isotope enrichment process described here was originally designed for a novel mass spectrometer system, following an idea of Bennett⁹. Bennett proposed a beam/foil conversion of C^+ at 30 keV to C^- , with mass filtering of both charge states, thus eliminating background due to the presence of ^{14}N (where the probability of forming metastable N^- is extremely low) and molecular species.

Other methods using small samples have demonstrated the advantages of accelerating C^- , stripping to C^{n+} , and accelerating to energies above 10 MeV where their detection signatures can be better identified^{10,11}. Another similar proposal is to accelerate C^{2+} in a cyclotron¹². These methods imply an initial beam current of the order of 1–50 μA , a final count of 10^4 on modern carbon, and a run time of an hour or so. Results with the tandem van de Graaff accelerator show that enrichment is not necessary for the dating of recent samples. This is clearly shown in the accompanying article¹³, which presents results of van de Graaff mass spectrometric detection of ^{14}C with an experimental arrangement that would be suitable for the operation of a small facility dedicated to ^{14}C dating. However, it is in extending the age to which samples can be dated that the technique of isotope enrichment will be most important. An enrichment of 100–

300 corresponds to a decrease of 37,000–45,000 yr in age; with the likely limit for direct measurement in the 50,000–100,000 yr region, a very significant extension to dating possibilities can be made. The integrity of the sample may perhaps be the ultimate limit; at 10^5 yr, contamination by 1 p.p.m. of modern carbon will double the ^{14}C content, or make the apparent age 6,000 yr younger. Fortunately with small samples (the minimum sample at this age to give 100 counts with the maximum total detection efficiency of 5% expected in the tandem van de Graaff is 50 mg) it is easier to ensure that the sample has been stringently purified. For example, that extracted collagen from bone has the appropriate (highly distinctive) amino acid decomposition. It has been pointed out¹² that a large error in the abundance determination for samples many half lives old leads to a quite acceptable error in the date, because of their exponential relation, and so 100 counts is more than adequate to estimate a reliable date, provided the background is low enough.

We therefore expect laser enrichment to make a major contribution enabling carbon dating to be reliably applied well into the last interglacial period, once the new mass spectrometric methods have been fully developed. Pre-enrichment on recent samples would permit faster and cheaper radiation dosimetry techniques to be used. In both cases the use of much smaller samples than hitherto possible will bring many new opportunities to archaeological and environmental age measurements.

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R. E. M. HEDGES

Research Laboratory for Archaeology,
Oxford University,
6, Keble Road, Oxford, UK

C. BRADLEY MOORE

Department of Chemistry,
University of California,
Berkeley, California 94720

Received 17 July; accepted 10 October 1978.

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Age of the Olby–Laschamp geomagnetic polarity event

SEVERAL excursions or reversals of the Earth's magnetic field throughout the normal polarity Brunhes period which started 690,000 yr ago, have been reported recently; these were summarised by Verosub and Banerjee¹. The first recent event to be reported was recognised by Bonhommet and Babkine² in lavas from the Chaîne des Puys (Massif Central, France) and was named the Olby–Laschamp magnetic event, but no precise date for this event has yet been obtained. An upper limit of 20,000 yr was suggested by K–Ar data³ and has led to attributing to this event several recently discovered young magnetic events⁴. However, subsequent thermoluminescence and K–Ar data^{5,6} indicate ages older than 30,000 yr. To clarify these age relationships I report here my dating of the Olby flow by the $^{230}\text{Th}/^{238}\text{U}$ radioactive disequilibrium method.

The $^{230}\text{Th}/^{238}\text{U}$ method is well adapted to dating volcanic rocks younger than about 250,000 yr. It was successfully used in obtaining the ages of samples of the Irazu volcano (Costa Rica)⁷

Table 1 U and Th concentrations and $(^{230}\text{Th}/^{232}\text{Th})$, $(^{238}\text{U}/^{232}\text{Th})$ activity ratios in Olby minerals

Minerals	^{238}U (p.p.m.)	^{232}Th (p.p.m.)	^{232}Th ^{238}U	$(\frac{^{238}\text{U}}{^{232}\text{Th}})$	$(\frac{^{230}\text{Th}}{^{232}\text{Th}})$
Total rock	2.07	8.36	4.03	0.744	0.780 ±0.010
Pyroxene	0.492	1.97	4.00	0.750	0.791 ±0.019
Px.					0.783 ±0.018
Plagioclase	0.523	2.29	4.38	0.685	0.864 ±0.017
Pl.					0.904 ±0.017
Magnetite	0.951	2.93	3.08	0.970	
Mt1 80–23 µm					
Magnetite	1.52	4.01	2.63	1.142	
Mt2 23–7 µm					

and of Etna (Sicily)⁸. The method is based on the following chronological equation:

$$\left(\frac{^{230}\text{Th}}{^{232}\text{Th}}\right) = \left(\frac{^{238}\text{U}}{^{232}\text{Th}}\right)(1 - e^{-\lambda t}) + \left(\frac{^{230}\text{Th}}{^{232}\text{Th}}\right)_0 e^{-\lambda t}$$

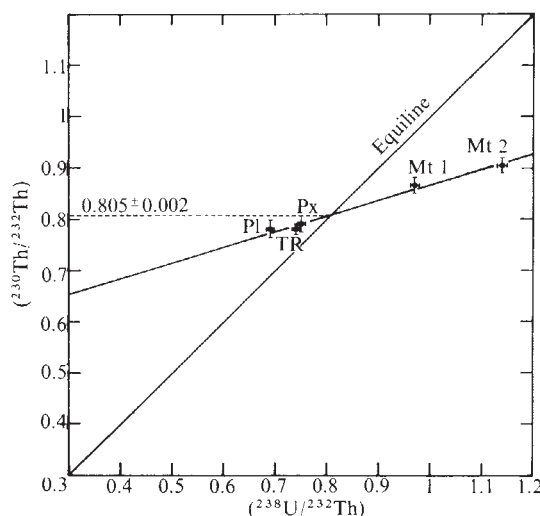
where the ratios are expressed in activity; λ is the ^{230}Th decay constant and ^{232}Th , considered as a stable isotope, is taken as a reference. The equation describes the return towards equilibrium between ^{238}U and ^{230}Th which has a characteristic period $T_{1/2} = 75,200$ yr.

Minerals of a volcanic rock crystallise from a magma with different $(^{238}\text{U}/^{232}\text{Th})$ ratios due to chemical fractionation between U and Th, but with the same $(^{230}\text{Th}/^{232}\text{Th})_0$ initial ratio if isotopic homogeneity is maintained. After a time t has elapsed their $(^{230}\text{Th}/^{232}\text{Th})$, $(^{238}\text{U}/^{232}\text{Th})$ define a line with slope $(1 - e^{-\lambda t})$ from which the crystallisation age may be obtained⁹. The $(^{230}\text{Th}/^{232}\text{Th})_0$ initial ratio is given by the intersection between the isochron and the principal bisectrix which is the position of the isochron when equilibrium is reestablished.

Details of the method and the analytical techniques are given elsewhere⁷. U and Th are measured by isotopic dilution and mass spectrometry. Errors in the $(^{238}\text{U}/^{232}\text{Th})$ ratios are estimated at 1%. Errors in the $(^{230}\text{Th}/^{232}\text{Th})$ ratios arise from counting statistics in α spectrometry measurements (1σ).

The sample studied is a K-hawaiite with olivine, clinopyroxene, plagioclase and titanomagnetite phenocrysts, from the inversely magnetised flow of Olby². The analyses were carried out on the total rock, clinopyroxene, plagioclase and two fractions of titanomagnetite of different grain size. The analytical results are reported in Table 1 and on Fig. 1. Least-square fitting of the data yield an age of $39,000 \pm 6,000$ yr and a $(^{230}\text{Th}/^{232}\text{Th})_0$

Fig. 1 Internal isochron of the Olby sample, $t = 39,000 \pm 6,000$ yr.



initial ratio of 0.805 ± 0.002 . Thermoluminescence dating of quartz pebbles of a palaeosol under the Olby flow yield an age of $41,000 \pm 4,000$ yr (ref. 10 and G. Valladas personal communication) which agrees well with my data.

Even though the duration of the Olby magnetic event is not known, most of the events found in the period 0–20,000 yr clearly cannot be attributed to the former. Denham and Cox have shown that if the Olby–Laschamp event had affected the entire Earth's magnetic field it could not have happened between 13,300 and 30,400 yr, or lasted less than 1,700 yr. Other magnetic field disturbances known in the period 30,000–50,000 yr are the Lake Mungo event¹² which lasted from 28,000 to at least 31,000 yr, the Lake Biwa excursion¹³ dated by extrapolation at 49,000 yr, an excursion the age of which is estimated at 40,000 yr in sediments from the Indian Ocean¹⁴, and, possibly, an event recently discovered in Iceland (the Maelifell event)¹⁵ which is not dated accurately yet. It is difficult to relate the latter events to the Olby–Laschamp one. If, however, the Lake Mungo and Olby–Laschamp disturbances represent the same event¹⁵ this must have lasted several thousands of years ($11,000 \pm 6,000$ yr). Correlation between young magnetic disturbances, whether local or global, and their interpretation must await accurate dating.

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MICHEL CONDOMINES

Laboratoire de Géochimie et Cosmochimie
et Département des Sciences de la Terre,
Université Paris VI et VII,
4 Place Jussieu 75230 Paris, France

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Phosphatic nodule beds in Victoria and the late Miocene–Pliocene eustatic event

CONTINENTAL evidence of the late Miocene expansion of the Antarctic ice cap and eustatic fall of sea level is still scarce. Here, phosphatic nodule beds in Victoria, Australia and other parts of the world are correlated with these events and with each other. Sedimentological and palaeontological evidence associates the Victorian beds with the late Miocene regressive phase and not with the succeeding early Pliocene phase of deposition. A fauna including whalebone, crabs and the nautiloid *Aturia*, accompanies the phosphatic nodules. The unusual abundance of phosphatic nodule deposits in the late Miocene is attributed to the contemporaneous evaporation, in the Mediterranean, of phosphate-deficient surface water derived from the North Atlantic, which created an enrichment in phosphate in the world ocean, of which the oversaturation surplus was precipitated. A

further association of the phosphatic nodule beds with areas of upwelling is postulated to account for their discontinuous occurrence.

Deep-sea evidence indicates that in the late Miocene, the Antarctic ice cap formed and grew to a larger size than the present one, possibly including an extensive partly floating and partly grounded ice shelf^{1,2}. Its growth is thought to have caused a eustatic fall in the sea level to at least 55 m below that at present¹. The late Miocene was the time of maximum regression^{1,3} and other events occurring in the higher southern latitudes, including the development of thermohaline circulation, the Antarctic circum-polar current³, a thermally uniform water mass and the Antarctic Convergence^{1,4}, all thought to have migrated northwards at the time of maximum glaciation. In the Northern Hemisphere adjacent to the British Isles, a submerged marine terrace indicating erosion by a low sea level in the Miocene has been reported^{5,6}.

At Hamilton in western Victoria⁷, a bed of calcareous clay containing phosphatic nodules rests unconformably on limestone and clay of early and middle Miocene age and contains abundant *remanié* foraminifera from both formations. At Minhamite, also in western Victoria⁷, nodules are distributed through sediment of similar type for up to 40 cm above the unconformity, but here the basement and the foraminifera derived from it are of middle Miocene age only. The rich foraminiferal and molluscan faunas at these localities establish the correlation of the beds above the unconformity with the Jemmys Point Formation in Gippsland, the stratotype of the Kalimnan Stage (early Pliocene). *Aturia coxi*, a species known elsewhere from the late Miocene, has been recognised in the nodule bed at Minhamite⁸.

At several localities in the Geelong district^{9,10}, phosphatic nodules occur on or immediately above an erosion surface which truncates marine sediments of early and middle Miocene age. Here they will be considered collectively. The sediment enclosing and overlying the nodules is a fine clay sand, containing a sparse, poorly preserved fauna of Kalimnan age. The Geelong area, alone of all the nodule bed localities considered here, lacks an abundant marine fauna in the formation above the unconformity. Fossil crabs occur frequently in the nodules themselves^{10,11}.

At Beaumaris, near Melbourne, a phosphatic nodule bed rests unconformably on middle Miocene clay and limestone and is overlain by 6 m of marine calcareous siltstones, above which another lithological and formational change occurs^{7,12}. As at Hamilton, the beds immediately above the basal unconformity contain *remanié* foraminifera of both early and middle Miocene age and also *remanié* megafossils¹³. Specimens of *Aturia coxi*, whose matrix suggests that they occur in the interval 0–40 cm above the unconformity, suggest that these lowermost beds are of late Miocene age⁸. Bivalve and gastropod faunas obtained mainly from the beds 0.4–6 m above the unconformity have been shown to have closest affinities with faunas from Kalimnan (early Pliocene) strata and not with those of Miocene age^{14,15}.

The recent discovery of a phosphatic nodule bed immediately below the Jemmys Point Formation in the Lake Tyers–Lakes Entrance area of Gippsland extends the known nodule bed occurrences a further 330 km eastwards and shows that the nodule bed horizon underlies the Kalimnan stratotype¹⁶. *Globorotalia puncticulata* occurs almost immediately above the nodule bed in this area. Although this species has been reported from the late Messinian in the Mediterranean area and elsewhere¹⁷, pre-Pliocene records are questionable¹⁸ and the species is not known below the Pliocene in New Zealand^{18,19}.

One concludes that these Victorian occurrences of the unconformable nodule bed represent an eroded sea floor at the time of the lowest retreat of sea level during the late Miocene regression and is the surface over the rising sea transgressed during the latest Miocene or earliest Pliocene. If the extinction of *Aturia* is truly a Miocene event and the erosion surface represents the lowest still-stand, this apparently occurred slightly before the end of the Miocene.

At each of the Victorian nodule bed localities referred to here, except Geelong, cetacean vertebrae, ribs, ear bones, teeth and other bone fragments occur more commonly than at any other locality in the Tertiary of Victoria. Where the unconformity below the nodule bed has eroded early Miocene deposits, sharks' teeth also occur in the nodule beds with moderate frequency. Chiu²⁰ has reported a bed of frequently occurring whalebones, together with sharks' teeth and crabs in Taiwan at a level stated to be exactly on the Miocene–Pliocene boundary. Whether the abundance of whalebone at this time in Victoria, Taiwan and off southern California²¹ (see below) is coincidental or indicates a peak in the abundance of whales at this time, is uncertain. Diastems at other levels in the Tertiary of Victoria do not concentrate whalebone.

Note that other well-known phosphorite occurrences, those on the South African continental shelf^{22,23}; Chatham Rise^{24,25} and Campbell Plateau²⁶ (east and south of New Zealand respectively); the phosphatic siderite deposits on the eastern Australian continental shelf²⁷ and those on the continental borderland of southern California^{21,28} have also been attributed to a process of 'phosphatisation of late Miocene sediments' (*auct.*). Important associates of the nodules at the off-shore localities of South Africa are *Aturia* sp. and whalebone²²; of southern California, whale and other marine mammal bones²¹ and on the continental shelf of eastern Australia, crabs occur in the nodules as they do in the Geelong district.

The formation of the nodules in the Geelong district was considered by Coulson⁹ to have been followed by a time of complete emergence from the sea, yet before deposition of the Kalimnan sands. Although Bowler¹⁰ did not support complete emergence, he agreed that the nodules formed entirely during the regressive phase. The nodules of Chatham Rise are also thought to have been completely emerged after their formation²⁵ and as the present water depth is about 500 m, it seems unlikely that this occurred as recently as the Pleistocene. Dating of the nodule beds at Beaumaris and Minhamite as late Miocene by the presence of *Aturia coxi*⁸, assigns them to the regressive phase and not to the early Pliocene transgressive phase. Evidence from other sources also dates the regression as late Miocene^{29,30}.

In South Australia, extending 300 km westwards of Hamilton, there are thin Pliocene deposits, usually resting disconformably or unconformably on older Tertiary or even pre-Tertiary rocks^{31,32}. Some of them are dated as post-early Pliocene, but collectively they represent the deposits of a transgressive phase suggestive of a eustatic rise in sea level and their faunas contain many genera of foraminifera and molluscs restricted to considerably lower latitudes at present¹⁵. The Wright Valley in Antarctica was glacially eroded and vacated by the ice before 4.2 Myr BP and subsequently invaded by the sea during the Pliocene³³. These observations are in accord with the raised southern ocean temperatures estimated for the early Pliocene³⁴ and apparently accompanying a eustatic rise in sea level.

A temporary northwards migration of the Antarctic convergence during the late Miocene glacial period could result in upwelling of Antarctic bottom water and consequent phosphate deposition on the Australian southern and eastern continental shelves, the Aghulas Bank, the Campbell Plateau and Chatham Rise. There seems little doubt that the upwelling of deep ocean water and phosphate deposition are closely associated³⁵.

In the Northern Hemisphere, circumstances which led to the formation of the Messinian evaporite deposits in the Mediterranean Basin^{36,37} provide a possible mechanism for increasing the amount of phosphate available for precipitation from the world ocean at that time. These evaporites formed from seawater entering the Mediterranean over shallow sills³⁶ and it has been estimated that they represent 6% of the total dissolved salts in the world ocean at that time³⁸. The supply of this evaporite material would have been drawn selectively from North Atlantic surface water, in which the phosphate content is less than in bottom and intermediate water in the Atlantic and

other oceans³⁹. The result would be a net increase in dissolved phosphate in deep water in all oceans, thus creating a situation in which increased phosphate precipitation in areas of upwelling could be expected to have occurred in the late Miocene.

A. N. CARTER*

Sedgwick Museum,
Downing St,
Cambridge, UK

Received 5 July; accepted 29 August 1978.

* Permanent address: School of Applied Geology, University of New South Wales, Kensington, New South Wales 2033, Australia

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Confirmation of ¹³⁷Cs dating by algal stratigraphy in Rostherne Mere

THE deep sediments of Rostherne Mere, Cheshire (National Grid Reference SJ 745843) offer a promising situation in which to study the relationship between biological stratification and radionuclide dating in the absence of gross bioturbation. The mere is small (46.5 hectare), but relatively deep (30 m maximum), and is reported to be largely devoid of benthic fauna in the persistently deoxygenated deep sediments¹. The sediments of Rostherne Mere contain the remains of a great variety of algae, in addition to diatoms. The preservation of non-siliceous algae is uncommon, although found in the sapropel deposits of the USSR². In Rostherne the empty spores of *Ceratium hirundinella* O.F. Mull., *Anabaena* species and *Aphanizomenon flos-aquae* (L.) Ralfs are recognisable, as are colonies of *Microcystis* species and cells of *Staurastrum* species. The phytoplankton of Rostherne Mere has been recorded at intervals since 1912 (refs 3–5) and sampled more frequently from 1962 (refs 6–8). The recent records (Table 1) show fluctuations in summer dominance, mainly between *Ceratium hirundinella* and *Microcystis aeruginosa* Kütz. emend., but blue-green algae

Table 1 Collected phytoplankton data 1962–77

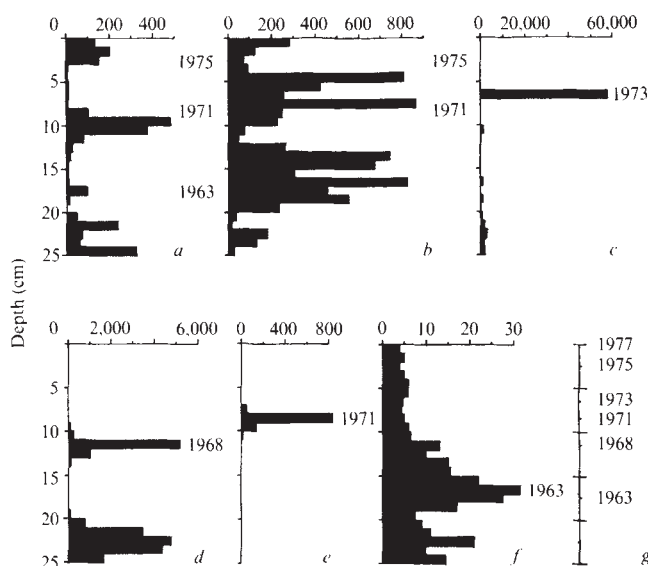
Year	<i>C. hirundinella</i>	<i>Microcystis</i> spp.	<i>S. astraea</i>	<i>M. granulata</i>	<i>C. pseudostelligera</i>
1977	Rare	Abundant	Rare	Rare	Rare
1976	No record	—	—	—	—
1975	Abundant	Rare	No record	No record	No record
1974	No record	—	—	—	—
1973	Common	Abundant	Abundant	Common	Rare
1972	Common	Abundant	Common	Common	Rare
1971	Abundant	Rare	Rare	Rare	Common
1970	No record	—	—	—	—
1969	No record	—	—	—	—
1968	Rare	Rare	Rare	Abundant	Rare
1967	Common	Common	Common	Rare	Rare
1966	Rare	Abundant	Common	Common	Rare
1965	Common	Abundant	Common	Common	Rare
1964	Common	Abundant	Common	Common	Rare
1963	Abundant	Abundant	Rare	Rare	Rare
1962	Rare	Abundant	Rare	Rare	Rare

Sources: 1962–63, ref. 6; 1964–66, ref. 7; 1967–77, ref. 8. No data available for 1976, 74, 70 and 69.

have become increasingly dominant⁹. These changes, combined with occasional maxima of the diatoms *Melosira granulata* (Ehr.) Ralfs, *Cyclotella pseudostelligera* Hust. and *Stephanodiscus astraea* (Ehr.) Grun., can be correlated with algal assemblages in the sediments and permit these to be accurately dated. We now report that such algal remains from the lake sediment have been used to establish a detailed chronology which is shown to confirm the ¹³⁷Cs dating method.

Three 1-m cores¹⁰ extracted from the deepest area of the mere were examined throughout for algal remains, and all showed the same well-defined stratigraphy. The algal species which are most numerous in the upper 25 cm of the cores are shown in Fig. 1 (a–e), and the patterns found reflect the changes in plankton dominance. Thus an 'algal chronology' can be constructed by comparing the sediment biostratigraphy with the observed and dated records of the plankton (Fig. 1g). The fact that the remains of many different algae are recognisable in the bottom deposits of Rostherne Mere permits a detailed correlation. Hence, the *Ceratium*-dominant years of 1963, 1971 and 1975 are allocated to the 17-cm, 9-cm and 3-cm horizons, respectively (Fig. 1 e,d).

Fig. 1 Algal and caesium stratigraphy. a, *Ceratium hirundinella* spores; b, *Microcystis* spp. colonies; c, *Stephanodiscus astraea* cells; d, *Melosira granulata* cells; e, *Cyclotella pseudostelligera* cells; f, caesium-137 pCi per section; g, algal chronology. a–c, Numbers expressed per 5 µl of fresh sediment, d,e, per 0.1 µl of fresh sediment. A 1-cm thick section of a core contains 29 ml of fresh sediment.



Two of the cores were dated by the distribution pattern of ¹³⁷Cs (refs 11,12). Sections 1 cm thick were dried and analysed by γ-ray spectrometry¹³ using a germanium (lithium) detector. The position of greatest concentration of ¹³⁷Cs, corresponding to 1963, occurred at a depth of ~17 cm in both cores and this demonstrates consistency between the cores and also agrees with the chronology established from the algal record. The ¹³⁷Cs profile from one of the cores is shown in Fig. 1 f. An earlier core dated by the ¹³⁷Cs method and reported by Gaskell and Eglinton¹⁴ showed about half the accumulation rate of the present cores. Such variations in accumulation rate at different locations are not unusual and have been discussed elsewhere¹⁵.

The deep sediments of Rostherne Mere have proved to be an ideal environment for the preservation of a variety of non-siliceous algae. Much of the sediment volume is derived from the high algal productivity of the mere, and these autochthonous deposits give a detailed insight into past phytoplankton communities. Also, the high rate of sediment accumulation (~1 cm yr⁻¹) ensures that a 1-cm slice provides adequate resolution of annual increments. The apparent absence of large benthic animals ensures that the ordered structure of the stratigraphic column is not likely to be grossly disrupted by bioturbation, and this is confirmed by the identical position of the ¹³⁷Cs peak in both the cores analysed.

Such conditions will not necessarily apply in other lakes, where there may be greater diffusion in the sediment column or more significant contribution from the catchment. Rostherne Mere is unusual because of the high autochthonous contribution to the sediment and small inflow of fine particulate allochthonous material. This is the first site in Great Britain at which independent evidence has been found for the validity of the dating of lake sediments provided by ¹³⁷Cs.

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DAVID LIVINGSTONE

Freshwater Biological Association,
Ambleside, Cumbria, UK

ROGER S. CAMBRAY

Atomic Energy Research Establishment,
Harwell, Oxfordshire, UK

Received 13 July; accepted 29 September 1978.

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Temporal decline in attractiveness of honeybee queen tracks

A BODY or surface (such as that of a worker bee or a cage) that has been in contact with a honeybee queen becomes attractive to worker bees^{1–3} due to contamination with queen pheromones^{3,4}. Little information is available about the durability of those pheromones, apart from Butler's report that 30 min after removal of the queen a cage becomes less attractive to the workers, and loses its attractiveness entirely after a further hour¹. I report here experimental evidence of a temporal decline in the attractiveness of tracks left on a waxed surface by a queen.

I monitored the accumulation of worker bees on a wire-gause cage which contained a queen^{4,5} or had been marked with her tracks on the inner surface. To achieve experimental conditions as near as possible to those inside a hive, the accumulation of bees was determined by weighing the cage with them on it. The cage was suspended from one of the arms of a balance and was let down, through a hole in the ceiling of the hive, over the brood chamber of the test colony. It was possible to put the queen into the cage and remove her from it without taking the cage from the hole. The cage was left over the brood chamber during the test, and the worker bees had easy access to it.

The cages used were covered with wax, and before the experiments they were kept in the hive of the test colony, which had a mated laying queen.

The bees that gathered on the cage containing a live mated queen were weighed. Then the queen was removed and weighing was repeated at 1-min intervals. The data obtained were used to give the relative attractiveness of queen tracks as a function of time (Fig. 1): it demonstrates a clear decline.

My results show that queen tracks can contain at least one pheromone that loses its effectiveness quite quickly, whereas the best known pure pheromone (9-oxodecenoic acid) has been reported¹ to remain detectably active in very small quantities for 7 yr.

The decline of attractiveness may occur either through volatilisation of the pheromone or in some other way. If queen tracks are assumed to form a thin film over a surface, the pheromone molecules in the film being dispersed far enough one from another (and consequently having an equal chance to leave the surface), the density of the molecules in the air near the surface (due to volatilisation) can be shown⁶ to change by the factor $\exp(-t/\tau)$ where t is time and τ is a positive parameter independent of t . The attractiveness of queen tracks can be expected to change in the same way. As Fig. 1 shows, the experimental data agree with this. Therefore the temporal change in attractiveness of queen tracks to worker bees is determined by τ ($27.9 \leq \tau \leq 29.2$ min for 95% confidence level). The half-life is 19.3–20.3 min.

Some evidence for a similar rate of loss of attractiveness of queen tracks is provided by Velthuis's observations of interactions between a group of queenless worker bees and "substitute queens" (workers bearing queen pheromones on their bodies). The attractiveness of the substitute queens lasted 5–45 min, the mean duration being 15 min (ref. 3).

Thus queen tracks are attractive to workers (and therefore are perceived by them) for sufficient time to have a possible influence on the behaviour of the workers and the state of the

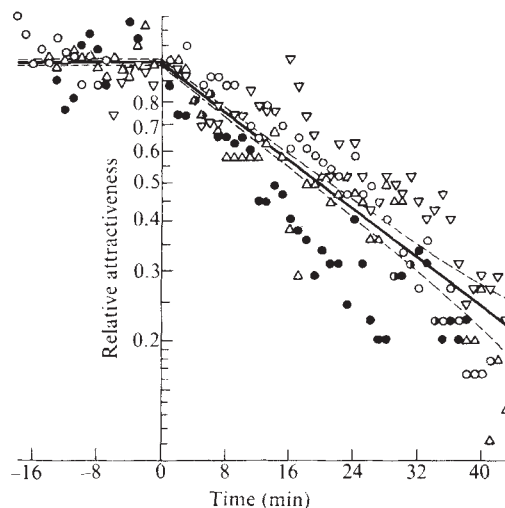


Fig. 1 Relative attractiveness to worker bees of a caged live mated queen (time < 0) and queen tracks left on the inner surface of the cage (time > 0). The different symbols correspond to data collected in four separate tests.

colony. On the other hand, the tracks lose their attractiveness (and cease to be perceived) sufficiently quickly to account for the rapid onset of queenless behaviour in a colony deprived of its queen.

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ALFONSAS JUŠKA

*Institute of Biochemistry,
Lithuanian Academy of Sciences,
Vilnius, Lithuania, USSR*

Received 5 November 1976; accepted 12 September 1978.

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The living coelacanth *Latimeria chalumnae* does not have a cloaca

POSSESSION of a cloaca is presumed to be a primitive vertebrate character that has been lost independently four times—in lampreys, chimaeras, actinopterygians and eutherian mammals—as the adult morphology of the urinary-genital-rectal openings is different in each group. Hagfishes, sharks, lungfishes, amphibians, reptiles, birds, monotremes, marsupials and *Aplodontia rufa* all possess a cloaca¹. Millot and Anthony² stated that the male *Latimeria* has a true cloaca whereas the female has a rectal opening separate from a common urinary-genital opening. We report here evidence that neither male nor female *Latimeria chalumnae* has a cloaca.

Male and female specimens of *Latimeria* were dissected to follow the digestive, urinary and reproductive tracts to their posterior openings. A plastic strip was inserted into the urethral

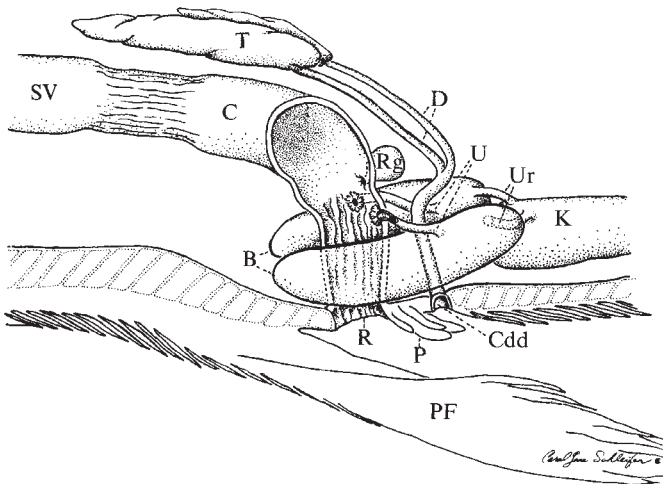
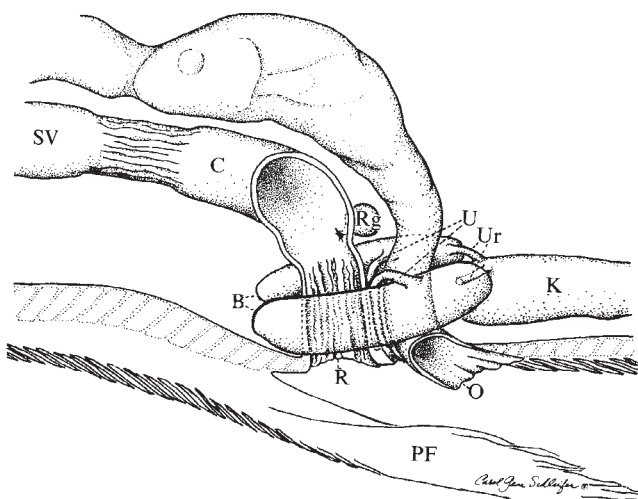


Fig. 1 Male rectal-urinary-genital anatomy of *Latimeria chalumnae*, lateral views, anterior to the left. Based on: AMNH 36941 (American Museum of Natural History) coelacanth 25 in the list of captured specimens³, 130 cm in total length, captured off Anjouan, Comoro Islands 10 October 1961, obtained from the Muséum National d'Histoire Naturelle, Paris; CAS 24862 (California Academy of Sciences) coelacanth 59, 122 cm in total length, captured off Itsoundzou, Grande Comore, Comoro Islands 1 January 1970; and LACM 6824-1 (Los Angeles County Museum of Natural History), 121 cm in total length, captured in the Comoro Islands in 1970 and not included in the list of coelacanth captures³. B, urinary bladder; C, colon; Cdd, common ductus deferens; D, ductus deferens; K, kidney; O, oviduct; P, papillary bodies; PF, pelvic fin; R, rectum; Rg, rectal gland; SV, spiral valve of the intestine; T, testis; U, urethra; Ur, ureter. (In using the above anatomical nomenclature we realise some would question the possibly implied homology between some of the osteichthyan and tetrapod structures. We do not necessarily imply such homology, but merely use the standard terms to name the structures.)

aperture in one of the urinary bladders and pushed through the urethra until it emerged from its distal opening. The other urethra in each specimen was slit open to follow the route of the urethra to the exterior. The oviduct of the female (AMNH 32949) had been opened previously, and was greatly enlarged due to the five advanced embryos it had contained. In the male (AMNH 36941), the left and right ducti deferentes were followed from the testes (the left testis was considerably larger than the right) to the point at which they anastomose, from whence a probe was passed down the common ductus deferens

Fig. 2 Female rectal-urinary-genital anatomy of *Latimeria chalumnae*, lateral view, anterior to the left. AMNH 32949, coelacanth 26; the specimen in which five advanced embryos were found⁴. See ref. 4 for the history of this specimen. Abbreviations as in Fig. 1.



to its external opening. The lower colon and upper rectum were slit open in the male; the entire length of the lower digestive tract of the female had been opened previously.

In the male, the urethras descend from the right and left urinary bladder and empty into the posterior wall of the rectum. A series of papillae surround the urethral openings where they join the rectum. The right and left deferent ducts descend from their respective testes and fuse into a common ductus deferens. From the point of fusion, the common ductus deferens descends

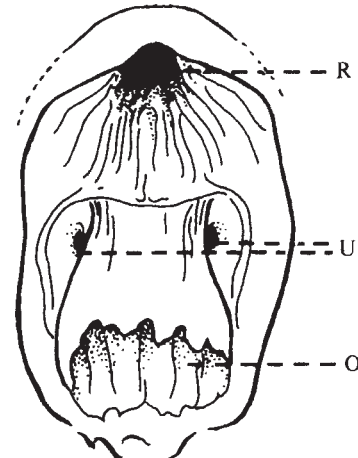


Fig. 3 Ventral view of rectal-urinary-genital openings of female *Latimeria chalumnae*, AMNH 32949. Anterior is to the top; abbreviations as in Fig. 1.

ventrally and opens externally (Fig. 1). Two additional male coelacanths (CAS 24862 and LACM 6824-1) also have the urethras opening high into the rectum without fusing with each other or with the two deferent ducts. In the female, the right and left urethras descend from the right and left urinary bladders and open directly to the outside on their respective antero-lateral sides of the oviduct. The oviduct itself opens directly to the exterior (Figs 2, 3).

We conclude that neither male nor female *Latimeria* has a cloaca and that the sexes are dimorphic: in the male the urethras empty into the rectum whereas in the female they open directly to the exterior. The anatomy of these openings in the female *Latimeria* is identical with that in many actinopterygians. However, the apparently plesiomorph condition for actinopterygians occurs in *Acipenser*, *Lepisosteus* and *Amia*, namely a rectal opening separate from a common uro-genital opening¹. This differs from the presumed plesiomorph condition for *Latimeria* which is maintained in the male as a common uro-rectal opening separate from the genital opening. We conclude that *Latimeria* exhibits another independent loss of the primitive cloaca.

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GUIDO DINGERKUS
HIN KIU MOK

Department of Ichthyology,
American Museum of Natural History,
New York, New York 10024

MICHAEL D. LAGIOS

Department of Pathology,
Children's Hospital of San Francisco,
San Francisco, California 94119

Received 7 July; accepted 21 September 1978.

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Morphological variance and enzyme heterozygosity in the monarch butterfly

It was shown several decades ago that the reduction in genetic heterozygosity produced by inbreeding in domesticated plants and animals is accompanied by an increase in morphological variability^{1,2}. While studies of laboratory populations and results of plant and animal breeding have been instrumental in identifying the relationship between genetic heterozygosity and morphological variability, such investigations often involved substantial changes in genetic heterozygosity, such as are produced when entire chromosomes are made homozygous. For this reason their relevance to the levels of heterozygosity in natural populations is unclear. Until recently attempts to examine this relationship in natural populations were hindered by the inability to routinely estimate levels of genetic heterozygosity in nature. However, determinations of genetic heterozygosity at specific enzyme-loci can now be made by electrophoresis. Recently Mitton³ found that heterozygotes for a set of five enzyme-loci had reduced multivariate variances for a set of morphological characters when compared to homozygotes at the same loci in populations of the killifish, *Fundulus heteroclitus*. It is very important to determine the generality of this observation for other species populations. I have now examined the relationship between genetic heterozygosity, as identified by electrophoresis, and the variance of morphological characters in the monarch butterfly, *Danaus plexippus*. Study of six polymorphic enzyme-loci in the monarch shows that once again heterozygotes have smaller variances for morphological characters when compared to homozygotes at the same locus.

The data for this study were gathered from over 1,400 monarch butterflies collected in the eastern USA and Canada during the summers of 1974–75. The sample sites, dates of collection, sample sizes, and electrophoretic methods are given in ref. 4. Six polymorphic enzymes were examined; glucose phosphate isomerase (GPI), malate dehydrogenase (MDH), glutamate-oxaloacetate transaminase (GOT), mannose phosphate isomerase (MPI), phosphoglucosmutase (PGM), and a TPN-dependent 'nothing' dehydrogenase (NDH). Electrophoretic variation at all six loci is inherited in a mendelian fashion, and it is known that the GPI, PGM and NDH loci are chromosomally unlinked. The chance of linkage between the other pair combinations is small.

Right forewing length and the diameter of the major forewing spot (divided by the length of the adjacent wing vein) were measured on each individual. These characters are normally distributed with a slight skew towards the smaller values. The correlation between characters is statistically nonsignificant ($r = 0.096$, $n = 54$). The characters are sexually dimorphic, and therefore males and females were treated separately in the analysis. There is very little geographic (sample) variation for either character; less than 2% of the total variance in either character can be attributed to between sample differences. Sample character variances are statistically homogeneous.

The association between genetic heterozygosity and morphological variability was examined by comparing the character variances of homozygotes and heterozygotes at each locus. Since there are two independent characters, six enzyme-loci, and both males and females to consider, this results in 24 statistical tests of the null hypothesis of no difference in character variances between homozygotes and heterozygotes. The results of those tests are given in Table 1. Because of the considerable differences in heterozygosity among the loci, and the smaller sample size of females, there is a substantial range of statistical power from test to test; the tests for GOT and MDH in females are the weakest.

The results show that homozygotes generally have larger variances for these morphological characters compared to heterozygotes at the same locus. The results are the strongest for

Table 1 Comparisons of morphological variances for enzyme homozygotes and heterozygotes in *Danaus plexippus*

Enzyme	Homozygote s^2	Heterozygote s^2	F_s	P
Character 1 (forewing length)				
Males				
PGM	3.35(371)	3.35(535)	0.97	0.639
MPI	3.68(426)	2.76(306)	1.33	<u>0.004</u>
NDH	3.09(730)	3.29(180)	0.94	0.710
GPI	3.35(492)	2.83(269)	1.16	0.081
GOT	3.16(877)	2.56(63)	1.23	0.110
MDH	3.16(866)	2.56(65)	1.23	0.110
Females				
PGM	4.07(153)	3.35(229)	1.21	0.091
MPI	3.71(209)	3.53(174)	1.04	0.388
NDH	3.64(310)	3.54(72)	1.04	0.450
GPI	3.85(203)	3.39(170)	1.13	0.193
GOT	3.68(364)	2.50(20)	1.47	0.150
MDH	3.63(354)	3.93(28)	0.92	0.650
Character 2 (forewing spot)				
Males				
PGM	0.033(382)	0.026(540)	1.27	<u>0.002</u>
MPI	0.030(471)	0.029(300)	1.03	0.406
NDH	0.029(734)	0.033(184)	0.88	0.847
GPI	0.031(501)	0.029(270)	1.07	0.265
GOT	0.033(877)	0.023(63)	1.44	<u>0.050</u>
MDH	0.028(866)	0.026(65)	1.09	0.323
Females				
PGM	0.033(165)	0.024(229)	1.38	<u>0.021</u>
MPI	0.032(222)	0.022(172)	1.46	<u>0.005</u>
NDH	0.029(319)	0.023(76)	1.26	0.124
GPI	0.030(217)	0.025(178)	1.20	0.084
GOT	0.028(374)	0.030(20)	0.93	0.631
MDH	0.029(366)	0.020(28)	1.45	0.110

Sample sizes are given in parentheses. F_s is the ratio homozygote s^2 /heterozygote s^2 . P is the probability associated with the one-tailed F test of the null hypothesis; σ^2 homozygotes = σ^2 heterozygotes. Forewing length variance is in mm^2 , and the forewing spot variance is dimensionless. Significant values underlined.

character 2. Overall, 19 of the 24 comparisons show homozygotes with larger variances, and five tests are statistically significant at the 0.05 level. None of the exceptions approach statistical significance. The distribution of test probabilities is noticeably skewed; 14 of 24 tests have probabilities less than 0.20. The results clearly support the hypothesis that heterozygotes at these loci tend to be found near the centre of the morphological character distributions.

It should be noted that some electrophoretic variants do not represent homogeneous allelic products, but instead are mixtures of different variants with similar electrophoretic appearance⁵⁻⁸. Consequently some heterozygotes will be misclassified as homozygotes, and this should tend to obscure the proposed association. The magnitude of this effect cannot be assessed in this study.

Problems certainly arise when assuming morphological variability to be a reasonable measure of developmental homeostasis. Morphological variability may predominantly reflect the segregation of additive and nonadditive genes rather than the expression of developmental instability. An association between enzyme heterozygosity and morphological variability could then be generated by electrophoretic variant alleles being in linkage disequilibrium with the genetic factors determining the morphological variance. It would be expected for electrophoretic heterozygotes to exhibit intermediate morphological values and smaller variances on the average. If this were the source of the observed association, then specific homozygous and heterozygous phenotypes should possess different mean values for the morphological characters. This was not the case. Of the 24 ANOVA tests performed to test for differences in

means between specific electrophoretic phenotypes, *none* were statistically significant. The ANOVA assumes equality of variances, a condition not satisfied here, however the test is robust to small deviations in homoscedasticity⁹. The outcome of these tests indicates that enzyme homozygosity affects the deviation from the morphological mean, and that the direction of that deviation does not depend on specific homozygous phenotypes.

The connection between genetic heterozygosity and developmental homeostasis led early investigators to propose that developmental buffering was enhanced by enzyme polymorphism along biochemical pathways¹⁰⁻¹², although few methods were available to assess levels of genic heterozygosity. The widespread use of electrophoresis in recent years and the discovery of extensive molecular polymorphism has prompted interest in the adaptive significance of enzyme polymorphism^{13,14}. Surprisingly little attention has been paid to the potential role of this enzyme heterozygosity in developmental homeostasis.

The results presented here suggest that genetic heterozygosity and perhaps enzyme heterozygosity *per se* are associated with developmental homeostasis in natural populations. However, the observation also involves a serious paradox. As each monarch butterfly is potentially heterozygous for thousands of such loci, all contributing in a similar fashion, it seems unreasonable to expect to observe this effect for any random set of six loci. Over the entire genome each individual should have approximately the same average heterozygosity, and the specific contribution of any single locus should be obscured. It must, therefore, be assumed that either these particular six loci possess unexpectedly strong contributions, or that they serve as indices of overall genetic heterozygosity. There is no reason to attribute special effects to these enzymes, but homozygosity could be associated across many loci, either through inbreeding (inbred individuals will be concurrently homozygous for many loci), or through linkage disequilibrium of large blocks of loci. No evidence can be found for significant levels of inbreeding in the monarch population⁴. There is already ample evidence from several studies in *Drosophila* that electrophoretic variants can be in strong linkage disequilibrium with linked chromosomal inversions¹⁵⁻¹⁹. Unfortunately nothing is known about the plausibility of inversion polymorphism in Lepidoptera.

The results of this study are important because they help to generalise the observation that morphological variability and genetic heterozygosity are related in natural populations. While it is not clear what role enzyme polymorphism of the type identified by electrophoresis plays in this association, it is apparent that natural selection, by removing the morphologically extreme, will favor heterozygosity at several of these enzyme-loci^{20,21}.

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WALTER F. EANES*

Department of Ecology and Evolution,
State University of New York,
Stony Brook, New York 11794

Received 31 July; accepted 26 September 1978.

*Present address: Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138.

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Humans and apes are genetically very similar

MAN'S closest living relatives are the great apes—the African chimpanzee and gorilla, and the Asian orangutan. Humans (*Homo*) are classified in the family Hominidae; chimpanzees (*Pan*), gorillas (*Gorilla*) and orangutans (*Pongo*) are classified in the family Pongidae. The gibbons, or lesser Asian apes (genera *Hylobates* and *Symphalangus*), are classified in the family Hylobatidae. All three families of humans and apes are included in the superfamily Hominoidea. Linnaeus recognised the basic morphological similarities between humans and apes by placing them together in the order primates (Anthropomorpha), although in separate genera. He did not use any category of classification intermediate between 'genus' and 'order'. However, when the 'family' category was introduced, humans and apes were placed in separate families. The prevailing view of modern taxonomists has been expressed by Simpson¹: "*Homo* is both anatomically and adaptively the most radically distinctive of all hominoids, divergent to a degree considered familial by all primatologists". With the methods of classical mendelian genetics it is impossible to make genetic comparisons between species which cannot be hybridised, as the presence of genes is ascertained by studying segregation in the progenies of crosses between individuals exhibiting different traits. However, advances in molecular biology have made possible comparisons between DNA sequences or, more often, between proteins from different organisms. If the proteins are identical in amino acid sequence, the genes coding for them are assumed to be identical. (This is not always true owing to the redundancy of the genetic code, but DNA nucleotide differences which change the encoded protein have greater evolutionary consequences than those which do not.) Many proteins from different organisms can be readily compared by means of gel electrophoresis. Although not all amino acid differences are detected by this method, it provides reasonably satisfactory estimates of the relative degree of genetic differentiation between closely related species. King and Wilson² studied electrophoretically 44 gene loci coding for proteins in humans and chimpanzees (*Pan troglodytes*), and found them to be about as similar as very closely related species of other organisms are to each other. We now report results confirming that finding and extend it to comparisons between humans and the other apes.

Using polyacrylamide and starch gel electrophoresis, we have studied 23 gene loci coding for blood proteins in humans (*Homo sapiens*), the two species of chimpanzee (*P. troglodytes* and the pygmy chimpanzee, *P. paniscus*), the gorilla (*Gorilla gorilla*), the Sumatran and the Bornean orangutans (*Pongo pygmaeus abelii* and *P. p. pygmaeus*), and three species of gibbons (*Hylobates lar*, *H. concolor* and *Symphalangus syndactylus*). Table 1 gives the genetic identity and the genetic distance for the comparisons between humans and each of these organisms. The genetic identity, *I*, may vary between 0 (complete genetic differentiation at every locus) and 1 (complete identity at every locus). The genetic distance, calculated as $D = -\log_e I$, may vary between 0 and ∞ , and estimates the number of electrophoretically detectable allelic substitutions that have occurred in the evolution of two species since their last common ancestor³. Table 1 also gives the number of individuals studied for each ape species. The 10 gorillas are unrelated wild-born animals; all other animals are either wild-born or unrelated to each other.

Humans are genetically about equally differentiated from all the great apes; on average, about 35.4 electrophoretically

Table 1 Genetic identity, *I*, and genetic distance, *D*, between humans and various apes

	No. of loci*	No. of individuals	<i>I</i>	<i>D</i>
<i>Pan troglodytes</i>	22	23	0.680	0.386
<i>Pan paniscus</i>	23	4	0.732	0.312
<i>Gorilla gorilla</i>	22	10	0.689	0.373
<i>Pongo pygmaeus abelii</i>	23	8	0.710	0.347
<i>Pongo pygmaeus pygmaeus</i>	21	3	0.705	0.350
<i>Hylobates lar</i>	21	4	0.489	0.716
<i>Hylobates concolor</i>	21	2	0.429	0.847
<i>Symphalangus syndactylus</i>	21	1	0.333	1.099
Averages†				
Great apes (1–5)	23	48	0.702 ± 0.009	0.354 ± 0.013
Lesser apes (6–8)	21	7	0.417 ± 0.045	0.887 ± 0.112
All apes (1–8)	21	55	0.595 ± 0.055	0.554 ± 0.105

* The proteins studied were adenosine deaminase, adenylate kinase, catalase, fumarase, glucose-6-phosphate dehydrogenase, glutamate oxaloacetate transaminase, haemoglobin $\alpha\beta$, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, NADH-diaphorase, phosphoglucosomerase, phosphoglucosomutase, 6-phosphogluconate dehydrogenase (red-cell proteins), albumin, alkaline phosphatase, ceruloplasmin, esterase, haptoglobin, leucine aminopeptidase (plasma proteins). The following are omitted: adenosine deaminase in the gorilla and the three lesser apes, catalase in the three lesser apes and fumarase in *P. troglodytes*.

† Arithmetic means with their standard errors.

detectable allelic substitutions have occurred for every 100 gene loci in the separate evolutions of man and each of the apes. As expected, the genetic differentiation is larger between humans and the lesser apes, but even here the degree of genetic differentiation is relatively small; on average, there have been about 88.7 allelic substitutions per 100 loci.

The significance of these results becomes apparent when they are compared with electrophoretic data obtained in other animal groups. The average genetic distances (calculated from published data, mostly in ref. 4) between pairs of closely related species are as follows: *Drosophila* flies, $D = 0.827$ (8 species); fish, $D = 0.622$ (4 species); salamanders, $D = 0.296$ (3 species); lizards, $D = 0.828$ (2 species); rodents, $D = 0.359$ (7 species). Although estimates of D are subject to large potential errors (typically, the standard error for a comparison based on 20–30 gene loci may be nearly half as large as the estimate), we may conclude that humans and apes (particularly the great apes) are genetically about as different from each other as closely related species of the same genus in other groups of organisms.

The genetic distance between man and *P. troglodytes* obtained by King and Wilson was $D = 0.62$, about 60% greater than the value obtained by us ($D = 0.386$). Although the two estimates are not significantly different from each other, it is worthwhile to examine the basis for such difference. King and Wilson studied 44 gene loci, whereas we have surveyed only 22 loci in these two species, 18 of which were included in their study. The difference in the two estimates of genetic distance could be accounted for, at least in part, if on average the loci surveyed by them but not by us were more different than the rest, or if the loci surveyed by us but not by them were less different than the rest. This is not, however, the case: when only the loci common to both studies are included in the estimation, the values of D remain approximately as different as for the complete samples. Therefore, the main reason for the higher estimate obtained by King and Wilson is that different electrophoretic methods were used in the two studies, and their methods must have allowed them to detect a greater number of differences.

Three observations should be made. First, the electrophoretic methods used with non-primate species are more similar to our methods than to those of King and Wilson (in fact, many of the studies have been carried out in our own laboratory); thus, our D value is more appropriately used when comparing the genetic differentiation between man and apes with that between other organisms. Second, the use of the D value obtained by King and Wilson does not alter the qualitative conclusion reached that man is genetically no more different from the great apes than are closely related (congeneric) species in other animal groups from each other. (And less different than genera of the same family

are in such organisms; for these, we have obtained the following D values: fish, 1.772; salamanders, 0.828; rodents, 1.833.) Third, electrophoretic methods do not detect all amino acid differences between proteins and thus underestimate genetic differentiation; however, this bias should be similar in primates and in other animals and therefore would not affect the validity of the conclusion reached.

The results presented here lead to a paradox. We perceive ourselves as being considerably different in morphology and behaviour from the apes, and this can hardly be exclusively attributed to the fact that humans more readily notice differences involving themselves (indeed, see the statement by Simpson¹ quoted above). However, we have obtained estimates of genetic differentiation between humans and the great apes no greater than, say, those observed between morphologically indistinguishable (sibling) species of *Drosophila* flies. One possible resolution of this paradox is to assume that the estimates are biased; there are thousands of structural gene loci and the few surveyed might not represent a random sample of the whole genome. This is indeed possible but there is no reason to suspect that the sample is biased. Another, more interesting alternative, has been suggested by King and Wilson² as well as by others^{5–7}. It may be that morphological and behavioural evolution involves primarily changes in gene regulation rather than changes in structural gene loci (that is, those coding for proteins). If this is so, substantial differences in genetic regulation would exist between humans and apes. We favour this alternative. Although this hypothesis is not supported by any direct evidence, it is favoured by some indirect evidence¹ as well as by the demonstration in *Drosophila* that adaptation may occur through changes in regulatory rather than in structural genes⁸.

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ELIZABETH J. BRUCE
FRANCISCO J. AYALA

Department of Genetics,
University of California,
Davis, California 95616

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Totipotent cells of parthenogenetic origin in a chimaeric mouse

IN strain LT/Sv mice, eggs often begin to develop parthenogenetically in the ovary after the first meiotic division¹⁻³. They undergo apparently normal cleavage and blastocyst formation, but at the egg cylinder stage they become disorganised and form teratomas. They persist as tumours composed of many kinds of tissue, indicating that cells of parthenogenetic origin are viable. Spontaneous parthenogenesis is also common in ovulated strain LT eggs. They cleave, form blastocysts and implant in the uterus, but die shortly afterwards, in the same way that embryos derived from experimentally induced parthenogenesis die early⁴⁻⁷. However, parthenogenetic cells have been 'rescued' by removing embryos from the oviducts of virgin females and grafting them to extrauterine sites such as testis¹ and kidney⁸. Those embryos become disorganised and can survive as teratomas composed of many kinds of viable tissues. Why, then, do cells of parthenogenetic origin fail to survive *in utero*? To investigate this problem we are using chimaeras derived from aggregates of eight-cell parthenogenetic and normal embryos², transferred to the uteri of pseudopregnant females. We report here that early embryonic parthenogenetic cells are totipotent. They can give rise to fully functional ova which, when fertilised by sperm, develop into normal individuals.

Parthenogenetic four- and eight-cell embryos of the pigmented strains LT/Sv and LTXBJ and their F₁ hybrids, and embryos derived from normally fertilised eggs of albino strain 129/Sv and (129 × A/He)F₁, were flushed from the oviducts of superovulated females. After removal of the zona pellucida with Pronase, one normal and two parthenogenetic embryos were aggregated in Whitten's⁹ medium in an atmosphere of 5% CO₂, 5% O₂ and 90% N₂ at 37 °C. The aggregated 'triplets' were transferred in groups of about 10 to the uteri of pseudopregnant (SJL × C57BL/10)F₁ females; 456 aggregates were transferred to the uteri of 47 females. Twenty-four offspring were produced and six were identified as chimaeras by their partially pigmented coats and irises (Table 1). Four male and two female partheno ↔ normal chimaeras were produced. All were mated with strain 129 albinos. The four males sired 285 offspring, all albinos. Chimaera no. 19 (129 × A/He)F₁ ↔ (LT × LT × BJ)F₁ produced 33 albino offspring. Chimaera no. 53 (129 ↔ LT) produced five litters of 29 albino offspring. Her sixth and last litter contained two albino and two (one male and one female) pigmented offspring (Fig. 1). The pigmented offspring could only have been derived from ova of parthenogenetic origin (LT) fertilised by normal albino (129) sperm.

We were able to identify the parthenogenetic origin (LT) of the ova of chimaera no. 53 by a genetic marker other than *C*, which determined the pigmented coats of her offspring. Strain

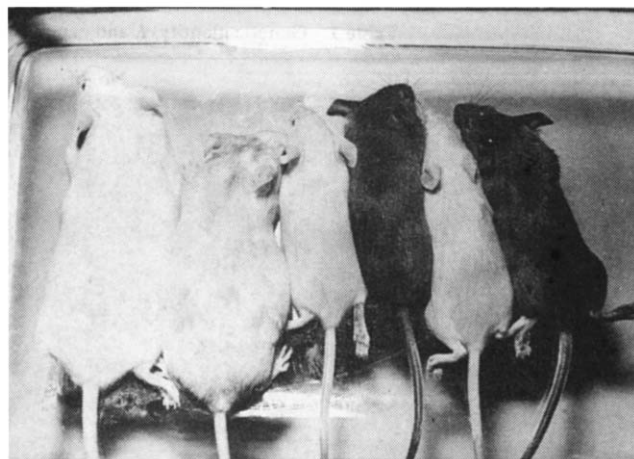


Fig. 1 An albino male (left) was mated to a chimaeric female (second from left) derived by aggregating normal and parthenogenetic 8-cell embryos. This mating produced a litter with two albino (derived from normal eggs) and two pigmented (derived from eggs of parthenogenetic origin) young.

LT is *Hbb*^s/*Hbb*^s, which produces an electrophoretically single haemoglobin, and strain 129 is *Hbb*^d/*Hbb*^d, which produces a diffuse electrophoretic pattern¹⁰. Cellulose acetate electrophoresis of mouse haemoglobins modified with the disulphide reagent cystamine permits rapid, unequivocal discrimination of all combinations of the co-dominant mouse haemoglobin 'single' (*Hbb*^s) and 'diffuse' (*Hbb*^d) alleles¹¹. Examination of the blood of the two pigmented offspring revealed that they were heterozygous *Hbb*^s/*Hbb*^d. The *Hbb*^s allele could only have been derived from ova of parthenogenetic LT origin.

Two of the chimaeric neonates were about half the size of their normal littermates. This suggested the possibility that in aggregates with parthenogenetic cells there is extensive cell death. To increase the possibility of having more parthenogenetic cells per embryo, parthenogenetic embryos were aggregated with other parthenogenetic embryos and transferred to the uteri of pseudopregnant females; 55 aggregates were transferred and five were recovered after implantation.

One female received nine aggregates of four parthenogenetic four-to-eight-cell embryos. Two abnormal embryos implanted; one had only a small amount of ectoderm undergoing necrosis and abnormally arranged parietal endodermal cells. The other was at the 6-d stage. It was larger than normal but the ectoderm was irregularly arranged.

Another female received 10 aggregates of three parthenogenetic embryos. There were three implantations; two were at the 5-d stage, with proamniotic cavities lined by dying ectodermal cells; the third was more advanced, but very abnormal. It had a primitive streak, chaotically arranged mesodermal cells,

Table 1 Production of chimaeras by aggregating normal and parthenogenetic embryos

Normal embryos (c/c)	Parthenogenetic embryos (C/C)	No. of aggregates transferred	No. of host females	No. born per litter	No. of chimaeras*
129/Sv	LT/Sv	160	16	2 born dead	?
				3	1 (53)
(129 × A/He)F ₁	LT/Sv	113	11	4	0
129/Sv	(LT/Sv × LTXBJ)F ₁	96	10	2	0
				4	3 (341, 42, 43)
129/Sv	LTXBJ	7	1	0	0
(129 × A/He)F ₁	(LT/Sv × LTXBJ)F ₁	80	9	2	1 (137)
				4	1 (19)
				3	0
		456	47	24	6

* Numbers in parentheses refer to designated chimaera no.

and an abnormal amnion, allantois and chorion. Although spontaneous parthenotes rarely develop to such an advanced stage, it is not certain that the advanced development was due to the increased number of cells produced by the aggregation of three embryos. These results suggest that increasing the number of cells in a parthenogenetic embryo will prolong its survival *in utero*.

Even though pure parthenogenetic embryos will not survive *in utero*, these results demonstrate that their cells are capable of participating in normal development when associated with normal cells. Furthermore, the production of offspring with the *C* and *Hbb*^s alleles from an aggregation chimaera between a parthenogenetic *C/C Hbb^s/Hbb^s* and a normal *c/c Hbb^d/Hbb^d* embryo demonstrates that fully functional totipotent ova of parthenogenetic origin were produced.

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Note added in proof: Illmensee¹² transplanted single ovarian teratocarcinoma cells into genetically different blastocysts and obtained chimaeric mice with internal organ mosaicism; in one case the tumour cells populated the germ line and developed into functional eggs, giving rise to healthy offspring.

LEROY C. STEVENS

The Jackson Laboratory,
Bar Harbor, Maine 04609

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Genetic regulation of glucose phosphate isomerase in mouse oocytes

THE enzyme glucose phosphate isomerase (GPI; D-glucose-6-phosphate-ketol isomerase EC 5.3.1.9.) catalyses the interconversion of fructose-6-phosphate and glucose-6-phosphate. In mice, the product of the GPI structural gene is a monomer and the enzymatically active dimer results from monomer aggregation^{1,2}. In inbred strains, GPI is found in either of two electrophoretically distinct forms: GPI-1AA or GPI-1BB (ref. 2). Theoretically, the random association of equal numbers of A and B monomers will result in dimers in the proportion of 25% AA:50% AB:25% BB, and indeed an activity distribution of these proportions is observed when samples of purified AA and BB forms, equal in enzymatic activity are dissociated and reassociated *in vitro*³. It follows, then, that (1) monomer aggregation is a random process which does not distinguish the monomer types; (2) the electrophoretic forms of GPI are similar enzymatically; (3) the measured activity is linearly proportional to the amount of enzyme. Therefore, the 25% AA:50% AB:25% BB activity distribution of GPI found in the mature tissues of heterozygous mice (*Gpi-1^{a/b}*) (ref. 4) indicates that the *a* and *b* alleles are equally expressed. However, we show here that this is not the rule for unfertilised ova, for we have observed differences in the expression of GPI in

Table 1 GPI activity in unfertilised ova

Female type from which ova obtained	GPI genotype	GPI activity (nmol per h per ovum $\pm$ s.d.)
DBA/2J	<i>Gpi-1^{a/a}</i>	0.36 $\pm$ 0.11
C57BL/6J	<i>Gpi-1^{b/b}</i>	1.59 $\pm$ 0.20
LP/J	<i>Gpi-1^{a/a}</i>	2.08 $\pm$ 0.22
B6D2F ₁ *	<i>Gpi-1^{a/b}</i>	1.07 $\pm$ 0.08
D2B6F ₁ †	<i>Gpi-1^{a/b}</i>	0.76 $\pm$ 0.08

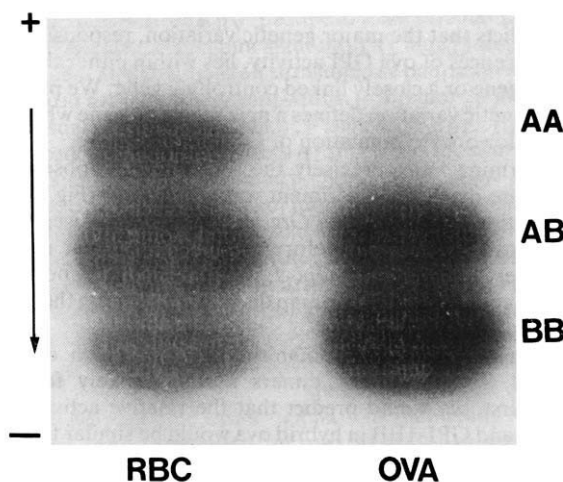
Ova were obtained from mature females in which ovulation had either occurred spontaneously or had been primed (3 IU pregnant mare's serum followed 45 h later with 3 IU human chorionic gonadotropin). 8–12 h post-ovulation, the females were killed and their ova were released into Brinsters medium (Gibco) by flushing the fallopian tubes. Adhering cumulus cells were removed by incubating the ova in hyaluronidase (Sigma type IV, 75 units ml⁻¹). The ova with intact zona pellucidae were washed three times in Brinsters medium. For spectrophotometry, the zona pellucidae were removed by incubation in Pronase (0.5%) followed by three washes in Brinsters medium. The ova were recovered either singly or in groups in approximately 1 μ l of medium in glass pipettes. Before analysis, these samples were stored at -80°C for up to 4 weeks. The activity of GPI was determined spectrophotometrically¹³ using pooled samples of up to eight ova. The assay mixture consisted of: 0.3 ml Tris HCl buffer, pH 8.0 (0.1 M Tris); glucose-6-phosphate dehydrogenase, type XII, from Torula yeast, 1 unit ml⁻¹; D-fructose-6-phosphate (Sigma, grade I) 4.65 mM; NADP, sodium salt, 2.25×10^{-1} mM. Three replicate assays per vum type were carried out at room temperature in a Guilford, model 250, spectrophotometer. The rate of the reaction was constant and linear during the assay period (25 min). All values, as assessed by the *t* test, are significantly different (<0.05) from their nearest neighbour. B6D2F₁ and D2B6F₁ ova were expected to have intermediate and similar activities. Ova from these reciprocal F₁ hybrids had GPI-1 activities between the two parental classes but they were not identical. The relatively small difference in GPI-1 activity between the two types of F₁ hybrids may result from a maternal effect, but this requires further investigation.

* B6D2F₁ female; progeny of C57BL/6J female and DBA/2J male.

† D2B6F₁ female; progeny of DBA/2J female and C57BL/6J male.

unfertilised ova from the inbred mouse strains DBA/2J, LP/J and C57BL/6J. Mature somatic tissues do not reveal these interstrain differences. We postulate that the amount of GPI in ova is controlled by a *cis*-active regulator gene linked to the GPI structural locus. This is apparently the first characterisation of a genetic variation affecting the amount of an enzyme in the

Fig. 1 GPI phenotype in red blood cells (RBC) and ova. Starch gel electrophoretogram⁴ demonstrating GPI expression within a heterozygous (*Gpi-1^{a/b}*) B6D2F₁ mouse. GPI in RBC demonstrates the expected proportions (25% AA:50% AB:25% BB) whereas ova demonstrate a pattern skewed in favour of GPI-1BB (3.4% AA:29.9% AB:66.8% BB). To assist in photography of the AA band, two identical ova were combined for this sample.



Parental strains	C57BL/6J	<i>Org</i> ^{high/high}	<i>Gpi-1</i> ^{b/b}	×	DBA/2J	<i>Org</i> ^{low/low}	<i>Gpi-1</i> ^{a/a}
Heterozygous F ₁ progeny	B6D2F ₁ <i>Org</i> ^{high/low} <i>Gpi-1</i> ^{b/a}						
	Ova phenotype: [A]:[B] = 21:79						
Backcross	C57BL/6J	<i>Org</i> ^{high/high}	<i>Gpi-1</i> ^{b/b}	×	B6D2F ₁	<i>Org</i> ^{high/low}	<i>Gpi-1</i> ^{b/a}
Heterozygous BC ₁ progeny	If recombination occurs <i>Org</i> ^{high/high} <i>Gpi-1</i> ^{a/b}			If no recombination occurs <i>Org</i> ^{high/low} <i>Gpi-1</i> ^{b/a}			
	Ova phenotype: [A]:[B] = 50:50			[A]:[B] = 21:79			
No. of progeny	0			65			

Fig. 2 Gene mapping study. The optimal circumstance for comparing the relative expression of the two *Gpi-1* alleles is to investigate their products in the same ova. The electrophoretic system used here permits that analysis (see Table 2). Therefore, ova from only *Gpi-1*^{a/b} backcross progeny were examined. The electrophoretograms (of the BC₁ ova) were scored visually. A recombinant would demonstrate the typical RBC pattern whereas a nonrecombinant would demonstrate the ova pattern presented in Fig. 1. *Org* represents the putative site responsible for the genetic variation of GPI activity in ova. From Table 1, we can designate *Org* as high for C57BL/6J and as low for DBA/2J.

mouse ovum and it provides a unique opportunity for further exploration of gene regulation during mammalian development.

Ova were obtained from the mouse strains DBA/2J, C57BL/6J and LP/J, and their GPI activities were determined spectrophotometrically. Table 1 shows that these ova express significantly different GPI activities, which do not seem to be correlated with the electrophoretic phenotype of GPI, as both LP/J and DBA/2J are homozygous for the same electrophoretic allele (*Gpi-1*^a). Nevertheless, relevant differences in the structural gene cannot be excluded on this basis alone, because the GPI structural genes in these strains could differ in ways which do not affect the electrophoretic mobility of the protein product.

As a first step in uncovering the mechanism responsible for these differences in GPI activity, ova from reciprocal F₁ hybrids between C57BL/6J (*Gpi-1*^{b/b}) and DBA/2J (*Gpi-1*^{a/a}) were examined. Although significantly different, their enzyme activities were found to be intermediate between those of ova from the parental strains (Table 1). In these F₁ ova, the expression of the two alleles may be under coordinate or individual control (see ref. 5). Coordinate control would lead to the equal expression of *a* and *b* alleles; if the two alleles were controlled individually, however, we would predict from the spectrophotometric data of the parental strains that the activity distribution would be skewed in favour of GPI-1B (Table 2). Figure 1 shows that the activity distribution is skewed. The ratio of A to B monomers is not unity but matches the ratio of the oocyte activity of the respective parental strains, indicating that in the maturing oocyte the *a* and *b* alleles are under individual control.

The electrophoretic distribution of GPI activity in all of 44 individual F₁ ova (16 ova from six B6D2F₁ females and 28 ova from seven D2B6F₁ females) was identical, indicating that the GPI phenotype of an unfertilised ovum is independent of both its prospective haploid genotype⁶ and its maternal origin. Therefore, our results are consistent with a *cis*-regulation model which predicts that the major genetic variation, responsible for strain differences of ova GPI activity, lies within either the GPI structural gene or a closely linked controlling gene. We propose that this genetic variation defines a new regulator gene which we name *Org* for oocyte regulation of GPI.

To determine more precisely the site of the proposed *Org* locus, a gene-mapping experiment was carried out (Fig. 2); no recombination event between *Org* and *Gpi-1* was observed in 65 backcross progeny. Therefore, if we assume that recombination between *Org* and *Gpi-1* is possible, the upper limit (with 95% confidence) for the map distance separating the two is 4.5 map units⁷.

Of the many possible mechanisms for the action of *Org*, differential degradation of dimers seems unlikely for two reasons. First, we would predict that the relative activities of GPI-1AA and GPI-1BB in hybrid ova would be similar to those observed between the ova of the parental strains (that is, 19:81) if a difference in the stability of the dimers, in the ova cytoplasm,

were involved. However, the electrophoretic activity distribution of GPI closely approximates that expected if the monomers were being produced in the ratio predicted from parental activities, and not that expected from degradation of dimers. The second reason is provided by the *Gpi-1*^c allele. Mice homozygous for this allele produce a GPI-1CC dimer which is electrophoretically distinct from GPI-1AA and GPI-1BB forms, and the GPI-1CC homodimer is unstable. The mouse red blood cell is expected to show little or no protein synthesis and therefore is a useful preparation for examining relative protein stability⁸. In the red blood cells of mice heterozygous for the *Gpi-1*^c allele, although the three expected bands are present, there is relatively little activity in the GPI-1CC band⁸. However, red blood cells from reciprocal hybrids between C57BL/6J and DBA/2J strains do demonstrate the typical 25%AA:50%AB:25%BB pattern (Fig. 1) which suggests that the dimers in these mice have equal stability.

Our findings describe a genetic variation affecting the expression of an enzyme during oogenesis in the mouse. Although the enzyme profile of unfertilised ova and preimplantation embryos from 'random bred Swiss' mice has been intensively studied^{9,10}, ova from inbred strains have not been similarly examined. In the only other study similarly comparing ova from different inbred

Table 2 Expression of *Gpi-1* alleles in F₁ ova

Ova obtained from female of genotype	Predicted ratio of A to B monomers, if <i>Gpi-1</i> ^a and <i>Gpi-1</i> ^b are		The ratio of A to B monomers determined by electrophoresis‡
	Coordinately* controlled	Individually† controlled	
B6D2F ₁	50:50	19:81	21:79
D2B6F ₁	50:50	19:81	18:82

* If coordinately controlled, both *Gpi-1* alleles would be equally expressed and therefore both A and B monomers will be present in equal amounts.

† From Table 1 the ratio of GPI activities of DBA/2J:C57BL/6J is 19:81. If individually controlled, the *Gpi-1* alleles would be unequally expressed and therefore A and B monomers will be present in unequal amounts, in the ratio of 19:81.

‡ Using a sensitive filter overlay technique for recovering an indirect reaction product of the GPI enzyme, it is possible to quantitate patterns on starch gel electrophoretograms⁴. The observed proportions (±s.d.) were, for B6D2F₁ ova: 5.3±1.5 AA; 31.8±3.0 AB; 62.9±3.4 BB and for D2B6F₁ ova: 3.4±1.7 AA; 29.8±3.0 AB; 66.8±4.5 BB. As the monomer compositions of the three GPI electrophoretic forms are known, it is possible to calculate the ratio of A monomers to B monomers for any given electrophoretogram. Let [A] and [B] represent the quantity of the respective GPI monomer types and [AA], [AB] and [BB] represent the amount of the dimer types, then the ratio of A:B monomers is ([AB]+2[AA]):([AB]+2[BB]).

strains, small but significant interstrain differences in lactate dehydrogenase activity were observed¹¹. Therefore, it seems that biochemical data on the ova of random bred Swiss mice may not be generally representative.

We conclude that the absolute level of ova GPI activity, although strictly regulated within each strain, can vary over a considerable range and yet permit successful reproduction. This situation now provides a unique opportunity for further exploration into developmental gene regulation during oogenesis and also during preimplantation development^{12,13}.

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A. C. PETERSON
G. G. WONG

Department of Neurosciences,
McMaster University,
1200 Main Street West,
Hamilton, Ontario, Canada L8S 4J9

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A better cell line for making hybridomas secreting specific antibodies

FUSION of myeloma cells which grow in tissue culture with spleen cells from an immunised mouse provides a general method for obtaining cell lines (hybridomas) which make antibody of the desired specificity^{1–3}. Hybrids derived from these myelomas make the immunoglobulin (Ig) heavy and light chains of the myeloma parent as well as the antigen-specific heavy and light chains of the spleen cell parent. In conditions in which the two heavy and two light chains associate randomly, a hybridoma would make 10 distinct Ig molecules, and the specific antibody would comprise only 1/16 of the total Ig^{4,5}. To obtain hybridomas making only the specific antibodies requires a tumour cell fusion partner that itself makes no Ig but which can nevertheless be fused with spleen cells to obtain hybrids secreting only the specific antibody. We report here the identification of such a cell line, Sp2/0-Ag14.

Sp2/0-Ag14 was isolated as a re-clone of Sp2/HL-Ag, itself derived in several steps from Sp2/HLGK, a hybrid between a BALB/c spleen cell contributing a γ 2b (H) and κ (L) chain with anti-sheep red blood cell activity and the myeloma cell line X63-Ag8 (γ (G) and κ (K))². Sp2/0-Ag14 is resistant to 20 μ g ml⁻¹ 8-azaguanine, dies in HAT supplemented medium and synthesises no Ig chains. It has about 73 chromosomes which is only eight more than the chromosome number of X63-Ag8, a cell line commonly used to generate hybridomas.

Sp2/0-Ag14 was fused with spleen cells of mice immunised with trinitrophenyl derivatised keyhole limpet haemocyanin (TNP-KLH) to generate hybridomas making TNP-specific immunoglobulin (Fig. 1). Fusions were done with either polyethylene glycol (PEG) 1500 or Sendai virus, and transferred to medium either containing or lacking mouse peritoneal exudate cells. Growth was observed in only eight culture wells, all from the set that were fused with PEG and grown with peritoneal exudate cells.

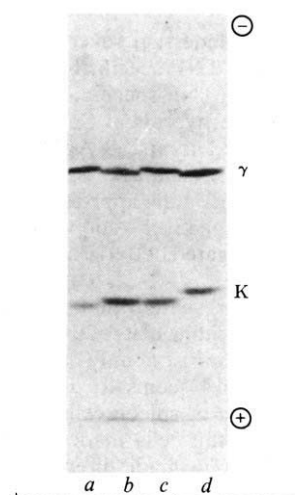


Fig. 1 Immunoglobulin from TNP-specific hybridomas analysed by SDS-polyacrylamide gel electrophoresis. To obtain hybridomas making Ig specific for the hapten trinitrophenyl, BALB/c mice were immunised i.p. with trinitrophenyl-derivatised keyhole limpet haemocyanin (TNP-KLH) (from H. Kiefer), first with 100 μ g TNP-KLH contained in 0.2 ml emulsion of 50% phosphate-buffered saline (PBS), 50% complete Freund's adjuvant (Difco). 35 days later a second injection of 100 μ g TNP-KLH in 0.2 ml PBS was made i.p., and 5 days later the spleens of these mice were prepared for fusion. Fusion with PEG 1500 was as described by Galfre *et al.*⁹, except that the medium used was R medium, RPMI 1640 medium (Gibco) supplemented with 30mM HEPES (Flow). Here 3×10^7 Sp2/0-Ag14 and 5×10^7 spleen cells were centrifuged together at 200 g for 5 min and resuspended slowly in 0.6 ml 50% PEG in Dulbecco's modified Eagle's medium (Flow). After 1 min at 37 °C, 20 ml of R medium was added slowly. The cells were then centrifuged and resuspended in 20 ml of R medium supplemented with 10% fetal calf serum (Gibco) (RF medium) and 0.2 ml of this suspension was then distributed to each of 200 wells containing 0.8 ml RF medium. One hundred of these wells also contained 2×10^5 mouse peritoneal exudate cells. After 24 h incubation, 1 ml RF supplemented with hypoxanthine, aminopterin and thymidine (HAT) was added to each well. Every 2–3 days, 1 ml of the medium was replaced with fresh RF+HAT. After 2 weeks, growth was observed in eight wells of the peritoneal exudate supplemented wells and the cultures were tested for immunoglobulin production by testing the culture medium for lysis of protein A coated sheep red blood cells (SRC) for nonspecific Ig¹⁰ and of TNP coated SRC² (for TNP specific activity). After 1–2 months the cells from TNP-specific wells were cloned by plating in soft agar¹¹ or in methyl cellulose¹². The soft agar clones were overlaid with TNP coated SRC, and clones lysing the TNP coated SRC were transferred to liquid medium. The cells were grown in the presence of ¹⁴C leucine to label secreted Ig which was analysed by SDS polyacrylamide gel electrophoresis as described¹¹. The hybridoma cell lines analysed were hy5.19 (a), hy2.15 (b), hy1.2 (c) and hy3.3 (d).

Supernatant of four of the eight growth-positive wells lysed protein A but not TNP-coated sheep red blood cells (SRC) suggesting that the Ig made by these cells did not have TNP specificity. The Ig secreted by these cells was analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and found to be IgM (data not shown). The remaining four growth positive wells lysed both TNP and protein A SRC and cells from these wells were cloned and analysed further, as described below. SDS-PAGE analysis suggested that these cell lines secreted IgG (Fig. 1). Ouchterlony analysis of culture supernatants using class-specific antisera (Litton Bionetics) indicated that hy1.2 makes an IgG2a, and that hy2.15, hy3.3 and hy5.19 make IgG1 and that all four TNP-specific hybridomas make a κ light chain. The SDS-PAGE analysis shown in Fig. 1 indicates that the clones can be distinguished by the mobility of the light or heavy chain, implying that these cell lines are making different antibodies.

We have examined the TNP-specific hybridomas for re-expression of the SRC-specific IgG2b of the progenitor cell line Sp2/HL-Ag14 by testing for SRC-specific plaques. No plaques

were found and we conclude that revertants constitute fewer than one in 5×10^4 of the TNP-specific hybridoma cells.

The karyotypes of the TNP-specific cell lines provide evidence that these cells are hybrids of Sp2/0-Ag14 and mouse cells. Normal mouse cells and Sp2/0-Ag14 contain 40 and 73 chromosomes, respectively. The TNP-specific cell lines hyl.2, hy2.15, hy3.3 and hy5.19 contain approximately 100, 105, 106 and 99 chromosomes, respectively, and we suppose that fusion yielded hybrids that segregated better growing cells that had lost 7–14 chromosomes.

Evidence has been presented that X63-Ag8 cells preferentially fuse with the dividing cells of the spleen^{2,6,7}, which for the B-cell compartment consist mainly of Ig-secreting, plaque-forming cells. Sp2/0-Ag14 seems to display the same preference. In the population of spleen cells used for this fusion, approximately 0.4% of the cells made indirect (IgG) TNP-specific plaques and 0.004% made direct (IgM) TNP plaques. Assuming that 1% of the spleen cells are plaque forming cells⁸ one would anticipate that about 40% of the hybridomas would make IgG with TNP specificity, as was observed. On the other hand, about 90% of the nonspecific plaque-forming cells of the spleen make IgM rather than IgG⁸, so that one would anticipate that among the nonspecific hybridomas, 90% would also make IgM, a figure that is consistent with our observation that all four nonspecific hybridomas examined made IgM.

To obtain high-titre preparations of the TNP-specific Ig, 10^6 TNP-specific hybridoma cells were injected intraperitoneally into BALB/c mice. All caused tumours and yielded ascites fluid with titres generally about 100 times higher than for the culture supernatants.

The efficiency of fusion with Sp2/0-Ag14 has been variable. In our early experiments, such as the fusion described above, we obtained fewer hybrids than we ordinarily obtain with X63-Ag8. However, in more recent experiments, fusions with Sp2/0-Ag14 have often been more efficient, in particular in fusions with lipopolysaccharide stimulated spleen cells or with large spleen cells (F. Melchers, Clark and G.K., unpublished observations). We consider that Sp2/0-Ag14 can yield as many hybrids as X63-Ag8.

Alternative methods exist for obtaining hybridomas that do not express the myeloma Ig. It has been possible to isolate re-clones that have lost the expression of the nonspecific heavy or light chains². This problem has been reduced by fusing with NSI-Ag 4/1, a derivative of X63 that makes the κ but not the γ 1 chain of the myeloma². We anticipate that Sp2/0-Ag14 will prove yet a better cell line for generating hybridomas making truly monoclonal antibodies.

MARC SHULMAN*

C. D. WILDE†

GEORGES KÖHLER*

*Basel Institute for Immunology,
Grenzacherstrasse 487,
Postfach, 4005 Basel 5, Switzerland

†MRC Laboratory of Molecular Biology,
Hills Road, Cambridge, UK

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Activation of latent Epstein-Barr virus by antibody to human IgM

EPSTEIN-BARR VIRUS (EBV) is the causative agent of most infectious mononucleosis¹ and is also associated with two human tumours, Burkitt's lymphoma (BL)² and nasopharyngeal carcinoma². Lymphoid cell lines of B-cell origin have been established from patients with these diseases, and from lymphocytes transformed *in vitro* by EBV³. In lymphoid cell lines the lytic viral cycle is usually repressed even though the cells carry multiple copies of the EBV genome. These cells therefore provide a valuable model for the study of latent EBV infection. Although EBV is ubiquitous, its association with human malignant disease is to a large extent both ethnically and geographically restricted² and it is therefore of interest to understand the host factors involved in the regulation of the expression of the latent EBV genome. In infectious mononucleosis, EBV infection is associated not only with a specific anti-EBV antibody² and T-lymphocyte response⁴ but also with an increase in nonspecific IgM production⁵. EBV-infected B lymphocytes produce polyclonal Ig (ref. 6) and the majority of established EBV genome-positive lymphoid cell lines possess surface IgM (ref. 7). We report here that treatment of several human lymphoid cell lines with antisera to human IgM activates the latent EBV genome to give a marked increase in EBV-specific early antigen (EA). Also, in some cell lines this treatment induces an increase in virus capsid antigen (VCA).

Treatment of BL-derived Raji cells with antiserum directed against human immunoglobulins increased the number of cells expressing EBV EA by up to 5,000-fold, to give 11% EA-positive cells determined by either direct or indirect immunofluorescence (Table 1), and confirmed by EA-specific complement fixation⁸. The fluorescence was specific for EA, as no positive cells were observed after staining with either control EBV-negative human serum, or with anti-VCA-positive EA-negative antiserum. EBV-specified antigens can be induced in lymphoid cell lines by halogenated pyrimidines^{9,10}, inhibitors of protein synthesis¹¹ or the tumour promotor 12-*O*-tetradecanoyl

Fig. 1 Induction of EA in cultures of Raji cells treated with various dilutions of antiserum. Cells were cultivated for 72 h with a particular dilution of antiserum in the presence of IUdR ($25 \mu\text{g ml}^{-1}$). The number of EA-positive cells was then determined on acetone-fixed smears by indirect immunofluorescence. None of the antisera had any effect on cell viability at the dilutions shown in the figure. ○, Sheep anti-total human immunoglobulins; ●, goat anti-human IgM; □, goat anti-human IgA; ■, goat anti-human IgG; △, goat anti-human IgD; ◇, goat anti-human IgE; ▲, IUdR alone ($25 \mu\text{g ml}^{-1}$).

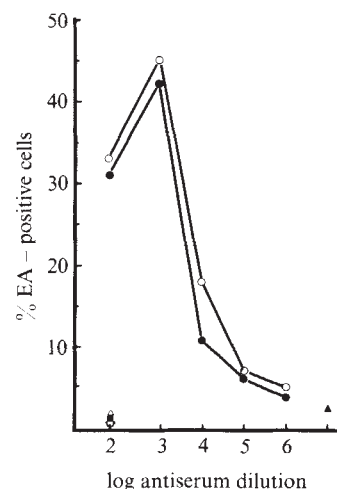


Table 1 The effect of anti-human immunoglobulins on the expression of EBV-specified antigens

Cell line	Expt no.	Time (h)	Antiserum dilution	% EA or (VCA) positive cells			
				Control	Antiserum treated	IUdR treated	Antiserum + IUdR treated
Raji	1	23	10 ⁻² Anti-total	0.002	4.5	0.1	21.0
		47	immunoglobulins	0.006	3.0	0.6	32.0
		71		0.014	7.0	0.7	36.0
	2	20	10 ⁻² Anti-IgM	0.002	11.3	—	—
	3	70	10 ⁻³ Anti-IgM	0.014	2.6	1.8	51.0
	4*	47	Anti-IgM antiserum	—	—	0.26	10.3
			Eluate	—	—	0.26	14.5
			Unbound material	—	—	0.26	0.35
	5†	22	10 ⁻³ Anti-IgM antiserum	0.014	4.8	0.016	10.0
	1	44‡	10 ⁻² Anti-IgM	0.12 (0.016)	9.0 (0.13)	10.0	21.0
Daudi	2	49‡	10 ⁻² Anti-total immunoglobulins	0.01 (0.004)	10.0 (0.36)	4.8	15.0
	1	23‡	10 ⁻² Anti-total immunoglobulins	0.002	0.032	0.004	11.0
BJAB-B95-8	1	23‡	10 ⁻² Anti-IgM	6.5 (5.5)	14.0 (7.0)	16.0 (14.0)	22.0 (17.0)
P3HR-1	1	47‡	10 ⁻² Anti-IgM	3.0	2.6	3.5	3.0
M61	1	44	10 ⁻² Anti-IgM				

Lymphoid cells were cultivated in RPMI 1640 medium with 20% fetal calf serum (Gibco) in static suspension culture for the times indicated with medium alone; medium + IUdR (25 µg ml⁻¹); the dilution of antiserum indicated; or IUdR + antiserum. EA or VCA was determined at this time in duplicate on 50,000 cells (fixed with acetone) either by direct immunofluorescence using FITC-conjugated EA(+) VCA(+), or EA(-) VCA(+) human sera, or by indirect immunofluorescence using EBV-specific human antisera and FITC-conjugated goat anti-human IgG globulin (Huntingdon).

* Anti-IgM antiserum was adsorbed on a column of immobilised human IgM as previously described³⁰. The serum and eluate contained an equivalent concentration of anti-IgM antibody determined by quantitative immunodiffusion³¹.

† In this experiment treatment of Raji cells with TPA (20 ng ml⁻¹, Sigma) gave 0.6% EA(+) cells and TPA with IUdR (25 µg ml⁻¹) gave 5.5% EA(+) cells.

‡ IUdR was removed by washing after 24 h treatment and the cells were then resuspended in either medium alone, or medium containing antiserum.

phorbol-13-acetate (TPA)¹². In optimal conditions treatment of Raji cells with antiserum consistently gave a greater degree of induction of EBV EA than treatment of these cells with either 5-iodo-2-deoxyuridine (IUdR) or TPA (Table 1). Treatment of Raji cells with antiserum in the presence of IUdR gave a synergistic effect resulting in up to 50% EA-positive cells (Table 1).

The active component of the antiserum to total immunoglobulins was found to be anti-human IgM immunoglobulin. Thus, the EA-inducing activity of antiserum to human IgM paralleled that of antiserum to total human immunoglobulin, whereas purified antisera to human IgG, IgA, IgE and IgD were all inactive (Fig. 1).

The activity of the anti-human IgM antiserum was specific for human IgM, as the EA-inducing activity of the antiserum was removed after passage through a column of immobilised IgM and could be recovered after elution of the column (Table 1). The effect of the anti-IgM antiserum was due to its anti- μ -chain activity, as antisera against the light chains λ or κ were inactive.

Normal human, sheep, goat and rabbit sera were all inactive as were antisera against human lipoprotein, β_2 macroglobulin and albumin. Raji cells have been reported to exhibit on their surface, in addition to IgM (ref. 13), a receptor for the Fc fragment of immunoglobulin¹⁴, a receptor for the C3 component of complement¹⁵, HLA histocompatibility antigens¹⁶, β_2 microglobulin¹⁶ and 'Ia'-like antigens¹⁷. However, no increase in EA-positive cells was observed when Raji cells were treated with complement (either alone or in the presence of anti-C3), anti- β_2 microglobulin antisera, specific anti-HLA antisera or specific anti-Ia antisera. Similarly, treatment of Raji cells with anti-human immunoglobulins in conditions which gave good induction of EA had no effect on the subsequent binding of IgG to the Fc receptor, or aggregated IgG in the presence of complement to the C3 receptor of Raji cells, as determined by the use of anti-human IgG labelled with ¹²⁵I-Staphylococcus protein A (ref. 18) (data not shown).

An EBV-specified DNA polymerase is thought to be one of the virus-coded proteins which constitute EA antigen¹⁹.

Table 2 Incorporation of ³H-thymidine and ³H-IUdR in Raji cells treated with anti-IgM antiserum

Labelled precursor	Sample	c.p.m. per 10 ⁶ cells	Specific activity (c.p.m. per µg DNA)	
³ H-thymidine	Control	227,811 ± 17,307	21,048 ± 1,869	NS
	10 ⁻² anti-IgM	208,288 ± 58,611	21,212 ± 955	
³ H-IUdR	IUdR	27,511 ± 2,515	12,798 ± 1,290	NS
	IUdR + 10 ⁻² anti-IgM	25,475 ± 3,310	12,319 ± 1,911	

Raji cells were cultivated in stirred suspension culture for 24 h with either 0.5 µCi ml⁻¹ ³H-thymidine (25 Ci mmol⁻¹, CEA) or 0.5 µCi ml⁻¹ ³H-thymidine + 10⁻² dilution of anti-IgM antiserum (Merieux), or 2 µCi ml⁻¹ ³H-IUdR (2.7 Ci mmol⁻¹, Amersham) + 25 µg ml⁻¹ cold IUdR, or 2 µCi ml⁻¹ ³H-IUdR + 25 µg ml⁻¹ cold IUdR + 10⁻² dilution of anti-IgM antiserum. Incorporation of labelled precursors into cellular material was determined by measuring the radioactivity in the trichloroacetic acid-insoluble material retained on a Millipore filter as previously described³². The specific radioactivity of purified DNA was determined after extraction of the DNA as previously described³³. NS, Not significant.

Treatment of Raji cells with anti-IgM antiserum, in conditions which gave a good induction of EA, also caused a marked increase in the activity of the virus-specified DNA polymerase (T. Ooka, G. L., M. G. T., unpublished results). Anti- μ antibody can activate B lymphocytes *in vitro*^{20,21}, leading to antibody production and the stimulation of ³H-thymidine incorporation. However, treatment of Raji cells with anti- μ antibody had no significant effect on either the incorporation of ³H-thymidine or ³H-IUdR into cellular acid-precipitable material or on the specific radioactivity of the purified DNA (Table 2), suggesting that EA induction by anti- μ antibody cannot be explained simply as a nonspecific mitogenic effect.

In agreement with previous reports²²⁻²⁵, no VCA-positive Raji cells were observed after short-term induction with IUdR or even in cultures treated with anti-IgM antiserum and IUdR, where 50% of the cells were EA positive. Our results suggest that the block in the abortive cycle of Raji cells is after EA and DNA polymerase synthesis and is independent of the level of induction. BL-derived Daudi cells, however, contain surface IgM and are capable of entering a lytic cycle. Treatment of Daudi cells with anti-IgM antibody markedly increased the number of both EA- and VCA-positive cells (Table 1). Treatment of the BL-derived BJAB-B95-8 cell line with anti-IgM antiserum gave up to a 2,000-fold increase in EA-positive cells, although no VCA-positive cells were observed either before or after treatment with antiserum. Some increase in EA (but not VCA) positive cells was obtained in anti-IgM-treated cultures of P3HR-1 cells (Table 1). Anti-IgM antiserum had no effect on the expression of EBV-specified antigens in the lymphoma-derived M61 cell line²⁵, one of the rare lymphoid cell lines which have surface immunoglobulin predominantly of the IgG class (Table 1). Thus, differences in the amount and/or expression of surface-bound IgM may partly explain variations in the response of different cell lines to the anti-IgM antibody, although the nature of the interaction between viral and cellular genomes is also probably important.

We have shown that anti-IgM antiserum is a more efficient inducer of EBV EA in Raji cells than either of the two most potent chemical inducers, IUdR or TPA, and the use of anti-IgM serum together with IUdR should facilitate the large-scale production of virus-specified antigens and DNA polymerase. However, the main interest of our results is that treatment of lymphoid cells with specific antisera against human IgM leads to activation of the latent EBV genome. The specificity of this interaction (that only anti-IgM antibody activates the EBV genome) suggests that this effect is probably mediated through an interaction of the antiserum with IgM on the cell surface. Activation may result either from a direct stimulation of virus replication by the antiserum, or from combination of the antiserum with surface-bound immunoglobulin which may play a part in the maintenance and perhaps even in the establishment of the latency of EBV infection in human lymphoid cells. It is perhaps relevant that the majority of these cells possess surface immunoglobulin of the IgM class⁷. EBV has been shown to infect B lymphocytes selectively²⁷ and to stimulate immunoglobulin production in these cells⁶. There is also evidence to suggest that EBV may preferentially transform IgM-carrying lymphocytes^{28,29}. It may well be that immunoglobulin production may in turn be related to the regulation of latent EBV infection in these cells. Our results, in addition to demonstrating a very efficient means of inducing EBV-specific antigens, may therefore have some bearing on the mechanism of the latency of EBV infection and its association with human disease.

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MICHAEL G. TOVEY

Laboratory of Viral Oncology,

GILBERT LENOIR

International Agency for Research on Cancer,
Lyon, France

JAQUELINE BEGON-LOURS

Laboratory of Viral Oncology,
Institut de Recherches Scientifiques sur le Cancer,
Villejuif, France

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Retinoids block phenotypic cell transformation produced by sarcoma growth factor

MURINE SARCOMA VIRUS (MSV)-transformed cells lack available receptors for epidermal growth factor (EGF)^{1,2}. We have shown that this altered phenotype is the result of the endogenous production of growth factors by the MSV-transformed cells themselves. The major activity, isolated and purified from transformed cells, has been found in a 12,000-molecular weight peak and competed with EGF in an EGF-receptor-binding assay³. This growth factor, called sarcoma growth factor (SGF), stimulates cell division, causes normal cells to grow in soft agar and produces rapid, reversible morphological transformation of cells in monolayer culture³. There is no evidence that SGF acts as a complete carcinogen, producing permanent cell transformation; its properties resemble classical chemical promoters of carcinogenesis, like 12-*O*-tetradecanoylphorbol-13-acetate (TPA)⁴⁻⁶, the highly active component of croton oil. Whereas TPA is an exogenous plant derivative acting on an animal or a cell, SGF is an endogenous,

Table 1 Effect of SGF and retinyl acetate on the final cell density of rat fibroblast cell cultures

Treatment	Cells per plate ($\times 10^{-6}$)	
	Expt 1	Expt 2
Untreated control	1.2	1.0
+ Retinyl acetate (1.9×10^{-8} M)	1.3	1.1
SGF-treated ($10 \mu\text{g ml}^{-1}$)	3.6	2.6
+ Retinyl acetate (1.9×10^{-8} M)	1.4	0.9
+ DMSO (1:1,000)	3.3	2.5

A subclone (536-7) of a normal rat fibroblast cell clone, NRK 49F (ref. 17), susceptible to the growth-stimulating effect of SGF (ref. 3), was seeded at 2×10^5 cells per plate in 60-mm plastic Petri dishes. The cells were treated at the time of inoculation and refed with medium (Dulbecco's modified medium with 1% fetal calf serum) containing the additions shown every third day. Cell counts were made 10 d after seeding. The SGF came from supernatants of MSV-transformed mouse NIH/3T3 cells as described previously³. Retinyl acetate was dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO in all experiments was 0.1%. Neither retinyl acetate nor DMSO alone showed any effect on the growth, final cell density or cloning efficiency of the normal rat fibroblasts at the concentrations used.

virally induced growth promoter. In this respect it is interesting that retinoids (vitamin A and synthetic analogues)⁷ block the action *in vivo* of exogenous and endogenous promoters, preventing carcinogens from producing new tumours, but do not reverse the growth of many established tumours⁷⁻¹⁰. Retinoids prevent cancer of the lung^{7,11}, skin⁹, bladder¹² and mammary gland¹³ in experimental animals, block cell transformation induced by chemicals¹⁴ and radiation^{14,15} in culture, and reverse the anchorage-independent growth of transformed mouse fibroblasts¹⁶. If SGF is part of the natural tumour-promoting system and retinoids are part of the natural defence against that system, then one should be able to demonstrate a direct antagonism in cell culture. This report establishes that this happens.

We have used a subclone (536-7) of a rat fibroblast cell clone (NRK 49F)¹⁷ that showed pronounced morphological transformation and anchorage-independent growth when treated with SGF, forming multiple cell layers and criss-crossing each other in an apparently random fashion. Although the effects are all reversible, these treated cells resemble MSV-transformed cells in their phenotype³. The cells which were treated with both SGF and retinoids did not have a disordered growth pattern. Table 1 shows that retinyl acetate, at 6 ng ml^{-1} , almost abolished the growth-stimulatory effect of SGF, as determined by the final cell density reached by the monolayer cultures 10 d after the experiment began. The effect of retinoids on SGF-induced morphological alterations was evident within a few days of treatment.

The retinoid concentrations ($1-2 \times 10^{-8}$ M) neither reversed the phenotype of virally transformed cells, nor blocked cell transformation produced by transforming viruses, such as the Moloney strain of mouse sarcoma virus (MSV) or simian virus 40 (SV40). Mouse 3T3 cells and rat fibroblasts were tested for susceptibility to transformation by MSV and by SV40. Neither retinyl acetate nor retinoic acid, up to 2×10^{-6} M, could be demonstrated to block either the initiation or the maintenance of virally induced transformation when efficient transforming viruses like MSV or SV40 were used (data not shown). In the same experiment, however, the SGF-induced morphological transformation was inhibited. Retinyl acetate did not inhibit normal cell growth or the cloning efficiency of the rat fibroblast cell clones in Petri dishes (data not shown), but did have a pronounced effect on the final cell ('saturation') density of cells treated with SGF (Table 1). SGF-induced cell growth was blocked and normal growth properties were essentially retained.

Table 2 shows that at concentrations well below those which produce any evidence of toxicity, retinoids prevent SGF-induced colony formation in soft agar. Colonies which did form

were smaller and contained fewer cells than those treated with SGF alone. Retinoic acid, retinyl acetate, retinyl methyl ether and retinylidene dimedone were all effective. Cells not treated with SGF and plated in agar remained as single cells with occasional 2-4-cell colonies. The clone used has a spontaneous transformation rate, as determined by agar colony growth, of less than one in 10^6 cells plated. The preparation of SGF used, at $10 \mu\text{g ml}^{-1}$, produced 40-50 large colonies per 10^4 treated cells and also many smaller colonies of 4-20 cells. The inhibitory effect of the retinoids was less evident or absent when more active SGF preparations or higher concentrations of SGF were used. Of the compounds tested, retinylidene dimedone was the most efficient inhibitor of SGF-induced phenotypic transformation (Table 2, expt 3). As a control against selective toxicity of retinoids to transformed cells as compared to normal cells, MSV-transformed mouse and rat cells which grow well in agar without SGF were plated in soft agar in the presence of retinyl acetate at 2×10^{-6} M. No reduction in colony-forming ability was seen.

These preliminary experiments establish that, in the system used here, retinoids block the transforming effect of the polypeptide hormone, SGF. Only one concentration of each retinoid, well below the level that produces any cell toxicity, was used, and both the growth promoter and the antagonists were added to the cells at the same time. This system is now available for further studies where the concentrations, duration of treatment and the nature of the interaction between each of the three components (promoter, antagonist and responding cell clone) can be varied in a systematic manner.

The general transformation model we propose has the following features. Viruses and chemical carcinogens act by inducing cells to produce normally repressed or inactive growth-promoting factors. These factors, which may be endogenous or exogenous to given cells, could be important in embryonic development, but if inappropriately expressed later in life could lead to transformation. Tumour viruses either provide transforming genes directly or activate cellular genes; chemical carcinogens do only the latter. These growth-promoting and

Table 2 Effect of SGF and various retinoids on the colony-forming ability of rat fibroblasts plated in soft agar

Treatment	Colonies per plate		
	Expt 1	Expt 2	Expt 3
Untreated controls	0	0	0
+ Retinyl acetate (1.9×10^{-8} M)	0	0	0
+ Retinoic acid (2.0×10^{-8} M)	NT	0	NT
+ Retinylidene dimedone* (1.5×10^{-8} M)	NT	NT	0
+ Retinyl methyl ether (2.0×10^{-8} M)	NT	NT	0
SGF-treated ($10 \mu\text{g ml}^{-1}$)	44.5	39.0	49.5
+ Retinyl acetate (1.9×10^{-8} M)	2.5	1.5	8.0
+ Retinoic acid (2.0×10^{-8} M)	NT	3.2	NT
+ Retinylidene dimedone (1.5×10^{-8} M)	NT	NT	0.5
+ Retinyl methyl ether (2.0×10^{-8} M)	NT	NT	14.5

On day 0, 1×10^5 rat fibroblast cells, clone 536-7 were treated in monolayer cultures using Dulbecco's modified medium with 1% fetal calf serum as described in the legend to Table 1. On day 2, they were seeded at 1×10^4 cells per plate in 0.3% soft agar containing the additions shown as described previously³. All cells not treated with SGF (whether treated with retinoid or not) remained as single cells with occasional (<10%) small colonies of 2-4 cells. Colonies with greater than 20 cells after 2 weeks in agar were scored as positive. NT, not tested.

* 5,5-dimethyl-2-retinylidene-1, 3-cyclohexanedione.

transforming factors may be produced during early embryogenesis and then 'switched off'. The endogenous viruses, with their capacity to recombine with cellular genes, have the ability to transfer information between cells and presumably within a cell, like bacterial insertion sequences. They may well be vehicles which allow expression of the endogenous growth-promoter structural genes. In this model the promoters, be they endogenous (SGF) or exogenous (TPA) act as proximal effectors of transformation.

In certain interpretations of the two-step theory of carcinogenesis, initiation involves gene damage^{4,18}; we suggest that this damage may facilitate the production of normally repressed growth factors and/or their cellular receptors. Exogenous promoters interact with specific membrane components and produce reversible phenotypic effects^{6,19,20}. Treatment of cells with either SGF or TPA causes a reduction in the number of available EGF – receptor sites on cells^{1,21}. SGF is a peptide hormone structurally similar to EGF; it is an acid- and heat-stable polypeptide with disulphide bonds and with an active component of a molecular weight 12,000 (ref. 3). The diterpenoid TPA (molecular weight 616), on the other hand, is structurally very different^{5,22,23}, and its effect on promoter-induced phenotypic change and/or competition for available EGF membrane-binding sites is not clear. Initiated cells may already be producing some of their own growth factors but not in sufficient quantity to cause phenotypic transformation. However, additional exogenous promoter would allow a critical concentration for transformation to be reached. A defined system, using cloned cells in culture to study natural tumour promoters and their antagonists, as described here, should permit a more precise analysis of the mechanisms of carcinogenesis.

The virogene-oncogene hypothesis²⁴ points out the possibly erroneous assumption that virally induced tumours would have to arise through external infection by emphasising that virus-coded or virus-associated genes are already present in several animal species. These genes, rather than the environmentally transmissible agents, are more likely to be involved in the origin of natural cancers. The tumour viruses, although unnatural, in that they have often been selected for producing rapid disease, have provided extremely powerful tools to dissect out and understand the molecular mechanisms involved. Genetically transmitted viral genes and transforming genes are now accepted as part of the normal genetic make-up of many organisms and as being activated by agents such as chemical carcinogens, hormones and radiation^{25,26}. In parallel with this is the frequently made assertion that chemical, environmental or even industrial carcinogenesis, are almost interchangeable with one another. The finding that SGF, produced by animal cells themselves, is an extremely potent promoter in cell culture systems suggests that endogenous growth promoters may be significant factors in naturally occurring cancers.

In view of the efficacy with which an endogenous factor such as SGF promotes transformation, natural mechanisms for suppression of transformation must exist. The data presented here, as well as previous *in vivo* studies, indicate that suppressors of transformation, such as retinoids, offer a potential approach to prevent the expression of the transformed phenotype.

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GEORGE J. TODARO
JOSEPH E. DE LARCO
MICHAEL B. SPORN

National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

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Retinoids as well as tumour promoters enhance deacylation of cellular lipids and prostaglandin production in MDCK cells

IN terms of a multiple-stage model of chemical carcinogenesis, tumour promoters may block differentiation of initiated (somatically mutated) cells and permit sufficient cell proliferation to overcome growth suppression^{1,2}. Deacylation of cellular lipids, arachidonic acid metabolism, or both, are biochemical pathways that accompany the effects of tumour promoters and may even mediate cellular differentiation and carcinogenesis³. While investigating the effects of several putative anticarcinogenic compounds, we found and report here that retinoids, like the tumour-promoting phorbol diesters, stimulated deacylation of cellular lipids and prostaglandin production in dog kidney

Table 1 Effect of retinoids on PGE₂ and PGF_{2α} production by MDCK cells

Retinoids	Concentration (μg ml ⁻¹)	No. of dishes	Prostaglandins produced (ng ml ⁻¹)	
			E ₂	F _{2α}
Control		10	0.28 ± 0.05	0.50 ± 0.05
TMMP	0.1	3	0.44 ± 0.02*	0.84 ± 0.08*
retinoic acid	0.5	3	0.58 ± 0.06*	1.40 ± 0.10*
	2.0	3	0.62 ± 0.03*	1.77 ± 0.25*
cis retinoic acid	0.5	3	0.42 ± 0.10*	0.75 ± 0.05†
	2.0	3	0.92 ± 0.05*	1.03 ± 0.18*
trans retinoic acid	2.0	3	0.62 ± 0.04*	1.27 ± 0.06*
Retinyl acetate	2.0	3	0.86 ± 0.07*	0.99 ± 0.06*
Retinol	2.0	3	0.76 ± 0.04*	1.00 ± 0.20*
Retinal	2.0	3	0.53 ± 0.04*	0.86 ± 0.07*
Retinyl palmitate	2.0	3	0.29 ± 0.02‡	0.57 ± 0.05‡

MDCK cells were incubated with the retinoids for 22 h. Each retinoid at the dose used does not cross-react with anti-prostaglandin antisera. Experiments with retinoids were carried out several times with qualitatively similar results. The values are the mean ± s.d.

* $P < 0.001$.

† $P < 0.01$.

‡ Not significant.

Table 2 Effect of retinoids and TPA on prostaglandin production and the release of radioactivity from ^3H -arachidonic acid-labelled MDCK cells

Treatment	Radioactivity (c.p.m. $\times 10^{-3}$ per dish)	Prostaglandins produced (ng ml $^{-1}$)		
		PGE $_2$	PGF $_{2\alpha}$	6-keto-PGF $_{1\alpha}$
None	19.9 $\pm$ 1.0	0.11 $\pm$ 0.04	0.66 $\pm$ 0.09	0.47 $\pm$ 0.12
+TPA	93.3 $\pm$ 6.4*	2.47 $\pm$ 0.35*	6.50 $\pm$ 0.66*	9.03 $\pm$ 0.93*
<i>cis</i> retinoic acid	36.6 $\pm$ 0.6*	0.38 $\pm$ 0.05†	1.15 $\pm$ 0.05†	2.13 $\pm$ 0.40*
+TPA	101.6 $\pm$ 1.7*	2.32 $\pm$ 0.21*	4.83 $\pm$ 0.59*	10.10 $\pm$ 1.77*
TMMP retinoic acid	43.2 $\pm$ 1.3*	0.64 $\pm$ 0.07*	2.60 $\pm$ 0.35*	4.23 $\pm$ 0.75*
+TPA	172.3 $\pm$ 1.4*	5.20 $\pm$ 0.58*†	12.67 $\pm$ 0.58*	22.67 $\pm$ 0.58*

MDCK cells were treated for 22 h with TPA (0.0005 $\mu\text{g ml}^{-1}$, Consolidated Midland), *cis* retinoic acid (2 $\mu\text{g ml}^{-1}$, Roche), TMMP retinoic acid (2 $\mu\text{g ml}^{-1}$, Roche) or their mixture. Prostaglandins produced were analysed by radioimmunoassay. Production of 6-keto-PGF $_{1\alpha}$ by MDCK cells was verified by high pressure liquid chromatographic analyses²¹. The values are the mean $\pm$ s.d. of triplicate dishes. The differences between 'none' and *cis* retinoic acid or TMMP retinoic acid are statistically significant. The differences between 'none' and TPA; *cis* retinoic acid and *cis* retinoic acid plus TPA; and TMMP retinoic acid and TMMP retinoic acid plus TPA are statistically significant ($P < 0.001$). The differences between TPA and *cis* retinoic acid plus TPA are not statistically significant. Those between TMMP retinoic acid plus TPA and TPA or *cis* retinoic acid plus TPA are statistically significant.

* $P < 0.001$. † $P < 0.005$.

(MDCK) cells. Treatment of the cells with a phorbol diester and a trimethylmethoxyphenyl analogue of retinoic acid led to a synergistic stimulation of lipid deacylation and prostaglandin production.

The MDCK cells were grown in Eagle's minimal essential medium containing 2 mM L-glutamate and supplemented with 10% (v/v) fetal bovine serum and penicillin-streptomycin. Cells were plated at 2×10^5 cells per dish and incubated in non-serum-supplemented medium with and without increments of retinoids and/or the phorbol diester for 22 h. Triplicate dishes were used for each reagent. Medium was assayed serologically for PGE $_2$, PGF $_{2\alpha}$, and 6-keto-PGF $_{1\alpha}$, with anti-PGE $_2$, anti-PGF $_{2\alpha}$ and anti-6-keto-PGF $_{1\alpha}$ antisera (the anti-PGE $_2$ antiserum reacts 0.01% with PGF $_{2\alpha}$ and the anti-PGF $_{2\alpha}$ antiserum reacts 0.1% with PGE $_2$, but the antisera do not identify the prostaglandins as either monoenoic or dienoic). The MDCK cells were labelled with ^3H -arachidonate as described previously⁴. They were extracted with 2:1 chloroform:methanol and the extract chromatographed on thin-layer silica gel G (Merck). Analysis of the radioactive materials showed that 69% of the radioactivity was associated with phospholipids, 15.0% with triglycerides, 11% with unidentified materials, 3.4% with prostaglandins and 1.4% with free arachidonic acid.

MDCK cells incubated in the presence of the trimethylmethoxyphenyl (TMMP) analogue of retinoic acid produced increased levels of PGE $_2$ and PGF $_{2\alpha}$. This stimulation was observed with as little as 0.1 $\mu\text{g ml}^{-1}$ (2.7×10^{-7} M) TMMP retinoic acid (Table 1). At the concentrations of retinoids used, the number of cells adherent to the culture dish was not affected, although higher levels of some of the retinoids ($\sim 3 \times 10^{-5}$ M), especially *cis* and *trans* retinoic acid and retinyl acetate, did detach cells from the dish. The stimulating activities of the different retinoids varied (Table 1). All the retinoids stimulated prostaglandin production, except retinyl palmitate, which, at levels $> 5.0 \mu\text{g ml}^{-1}$, inhibited prostaglandin production. However, this inhibition could be accounted for by its content of the phenolic antioxidant, butylated hydroxyanisole, used as preservative, which inhibits prostaglandin production (K.O. and L.L., unpublished data).

The synthesis of the prostaglandins by MDCK cells is limited by the availability of the precursor polyunsaturated fatty acids⁵. Stimulation of deacylation of the cellular lipids was demonstrated by the release of radioactive materials from ^3H -arachidonic acid-labelled cells treated with *cis* retinoic acid or TMMP retinoic acid (2.0 $\mu\text{g ml}^{-1}$, Table 2). Levels of PGE $_2$, PGF $_{2\alpha}$ and the prostacyclin non-enzymatic product, 6-keto-PGF $_{1\alpha}$, were also increased. The tumour-promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), was even more effective than the retinoids at stimulating release of radioactive materials and enhancing prostaglandin and prostacyclin production. The stimulating effects of both TPA and TMMP retinoic acid were more than additive; they acted in a synergistic manner. The

effects of *cis* retinoic acid and TPA were not synergistic; in fact, their combined effect was slightly less than additive.

Studies of the effects of vitamin A and its analogues on carcinogenesis have led to conflicting results. On the one hand, they modify skin tumour promotion and prevent epithelial cancers⁶⁻¹⁰. Topical applications of retinoids inhibit skin papilloma formation and the tumour-promoting phorbol diester induction of ornithine decarboxylase activity¹¹. However, enhancement of the effects of the chemical carcinogens, similar to that of the tumour-promoting phorbol diesters, has also been described¹²⁻¹⁵. With respect to cultured cells, pathways leading to membrane glycosylation may involve vitamin A as a lipid intermediate^{16,17} and vitamin A can alter surface membrane glycolipids and glycoproteins¹⁸. Retinoic acid inhibits the comitogenic action of TPA in bovine lymphocytes¹⁹, and retinoic acid and retinoids, at low concentrations, stimulate plasminogen-activator synthesis in chick embryo fibroblasts; at suboptimal levels of TPA, the effects of retinoic acid and TPA are synergistic²⁰. Clearly, the biological properties of the retinoids are manifold and their promoting or anticarcinogenic properties may depend on the type of membrane change induced, which probably in turn depends on the cell and the particular synthetic retinoid used. We suggest that the use of vitamin A and some of its analogues to prevent chemical carcinogenesis is premature; they may enhance, not inhibit tumour formation.

Unless indicated, the retinoids were from Sigma.

LAWRENCE LEVINE

KAZUO OHUCHI

Department of Biochemistry,
Brandeis University,
Waltham, Massachusetts 02154

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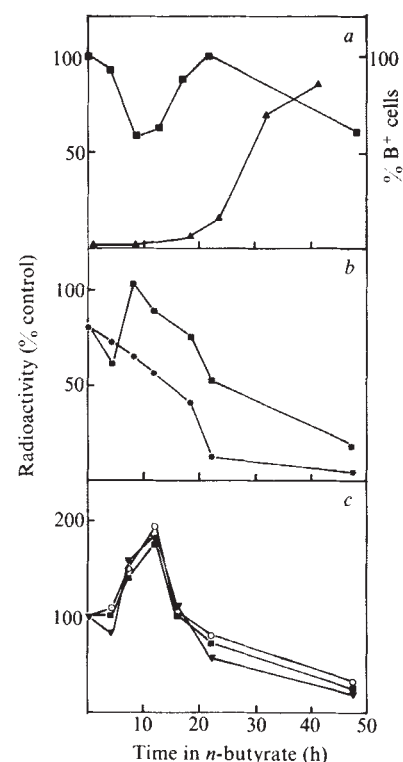
Uncoupled synthesis of histones and DNA during Friend cell differentiation

ERYTHROLEUKAEMIC mouse spleen cells (Friend cells) can undergo erythropoietic differentiation *in vitro* when cultured in the presence of a variety of different inducers¹⁻⁶. Untreated Friend cells are arrested at a proerythroblast-like stage of development in the erythropoietic lineage⁷ and contain little or no globin mRNAs and no haemoglobin. In response to an inducer the cells start transcribing the globin genes, accumulate globin mRNAs and haemoglobin in the cytoplasm^{3,8} and express morphological and biochemical changes which parallel changes observed during normal *in vivo* erythropoiesis in the mouse. Dimethyl sulphoxide-induced Friend cells (strain 745 A) show a transient block in the initiation of DNA synthesis which precedes detectable expressions of the differentiated functions⁹. We report here a G1 phase prolongation in another Friend cell line using another inducer (*n*-butyrate). We have also characterised the induction process in respect of the capacity of the cells to synthesise and transport proteins to the nucleus. The rate of synthesis of total nuclear proteins decreases steadily but the histones show a reproducible pattern of an initial increase in this parameter at a time when DNA synthesis is blocked, indicating that histone synthesis becomes independent of DNA replication during Friend cell differentiation.

A series of cultures was prepared starting from a single culture of F4N cells¹⁰ in exponential growth. *n*-Butyrate was added and at various times thereafter cells from one culture were collected for determination of cell density, incorporation by whole cells of

[methyl-³H]thymidine into acid-precipitable material and appearance of radioactive label in total nuclear proteins and in histones after labelling of cells with ³H-lysine and ³H-arginine. The results of such an experiment are shown in Fig. 1. In Fig. 1a cellular DNA synthesis after addition of *n*-butyrate is related to the percentage of haemoglobin-containing, benzidine-reactive (B⁺) cells⁹. For 4 h after addition of inducer the rate of DNA synthesis was unchanged but between 4 and 8 h after addition DNA synthesis decreased to half the original rate. This low-level DNA synthesis continued until 12 h after addition of inducer, when the rate of DNA synthesis increased to levels characteristic for exponentially growing cells; this period was followed by a final cessation of DNA synthesis. A significant increase in the number of haemoglobin-producing cells was observed only at about 20 h after the onset of *n*-butyrate treatment. The DNA synthesis curve and cytofluorometric determination of the DNA content of single cells suggest that an early effect of the induction process is a transient prolongation of the G1 period of the cell cycle resulting in an extensive depletion of S-phase cells¹¹. A similar prolongation has been described for dimethylsulphoxide-induced Friend 745A cells⁹ although the time scale for the changes is different. *n*-Butyrate acts much more rapidly than dimethylsulphoxide in inducing the expression of the differentiated function as monitored by the appearance of B⁺ cells. In accordance with this the prolongation of the G1 phase in our system is expressed both earlier and is of shorter duration than the prolongation observed with dimethylsulphoxide⁹. The G1 phase prolongation seems to be an inherent feature of the commitment process and might be necessary for the synthesis of specific regulatory substances required during the following S phase. This first S phase in the presence of the inducer seems to be unusual in some respects: the activity of the nuclear enzyme poly (ADP-ribose) polymerase is two- to fourfold greater, and

Fig. 1 Rate of DNA synthesis and appearance of newly synthesised proteins in the nuclei of *n*-butyrate-treated F4N cells. Ostertag's erythroleukaemic mouse spleen cell line F4N was used² and cells were kept in culture conditions described previously¹⁰. For induction of the erythropoietic differentiation 1 mM *n*-butyrate was added to culture medium supporting exponentially growing cells ($2-3 \times 10^5$ cells ml⁻¹) and cells were kept for various periods in the presence of the inducer. **a**, Incorporation of ³H-thymidine into DNA (c.p.m. per 10³ cells) (■) and appearance of benzidine reactive (B⁺) cells (▲). For the measurement of DNA synthesis 1-ml aliquots of cells that had been kept in *n*-butyrate for the time shown on the abscissa were incubated with 3 μ Ci [methyl-³H]thymidine (50 Ci mmol⁻¹) for 20 min at 37 °C. Trichloroacetic acid (TCA) precipitable material was determined by liquid scintillation counting. The percentage of benzidine reactive cells was determined using the suspension staining technique¹⁹. Between 200 and 300 cells were counted for each determination. **b**, The appearance of newly synthesised proteins in the nuclear fraction of *n*-butyrate-treated cells (total nuclear proteins, *; histones, ◆) expressed as per cent of untreated, exponentially growing cells was determined as follows. In sterile conditions $3-5 \times 10^7$ cells were collected, washed once with 5 ml lysine- and arginine-free culture medium, resuspended in 3 ml of the same medium which was substituted with 50 μ Ci ³H-lysine (74 Ci mmol⁻¹) and 50 μ Ci ³H-arginine (18 Ci mmol⁻¹). Incubation was at 37 °C for 150 min and the reaction stopped by chilling on ice; the cells were collected and washed once with 5 ml cold phosphate-buffered saline (PBS, 0.14 M NaCl, 2.7 mM KCl, 1.47 mM KH₂PO₄, 8.1 mM Na₂HPO₄), containing 1 mM phenylmethylsulphonylfluoride (PMSF). All subsequent buffers and solutions contained 1 mM PMSF to prevent proteolytic degradation. The cells were resuspended in PBS, PMSF and lysed with 0.15% Kyro EOB (Procter and Gamble). After 10 min at 4 °C no intact cells were detected as judged by Trypan blue staining. The released nuclei were pelleted at 1,000g for 15 min and suspended in 20 volumes of 0.15 NaCl, 50 mM Tris-HCl, pH 7.5, 1 mM PMSF. The proteins in this nuclear suspension were referred to as total nuclear proteins. The nuclei were pelleted again and washed twice more with 20 volumes of the same solution. At this stage the nuclei could be stored frozen at -20 °C for several days before histone extraction. Histones were extracted from the washed nuclei using 0.125 M H₂SO₄, 1 mM PMSF. Nuclei were suspended in 10 volumes of this solution with a Vortex mixer and left for 1 h in an ice bath with occasional stirring. The suspension was centrifuged for 20 min at 10,000g at 4 °C, the supernatant was taken away and the pellet was extracted in the same way once again. The two supernatants were combined and referred to as total histones. Determination of the incorporation of the radioactive amino acids into total nuclear proteins and into histones: aliquots of the nuclear suspension in 0.15 M NaCl, 50 mM Tris-HCl pH 7.5, 1 mM PMSF and of the acid extracted histones were precipitated with 20% TCA and were left for 30 min on ice. The protein precipitate was collected on Millipore filters and washed four times with 5 ml cold 12% TCA. The radioactivity on the filters was determined by liquid scintillation counting. **c**, Incorporation of ³H-lysine and ³H-arginine into histone H1 (▼); histones H3, H2a, H2b (■); and histone H4 (○). The histone classes were separated by SDS-polyacrylamide electrophoresis¹⁵, the Coomassie blue stained protein bands cut out, the proteins extracted from the gel slices with 0.5% SDS and the radioactivity in the extracts determined by liquid scintillation counting.



the extent of release of Friend virus sixfold greater than the levels observed in cells in exponential growth phase¹².

General protein synthesis gradually increases during the cell cycle, with the notable exception of histones. Synthesis of histones is restricted to the S phase of the cell cycle and is correlated with the synthesis of DNA¹³ suggesting that the chromosome as a whole and not just its genetic material is reproduced during S phase. As *n*-butyrate treatment changes the course of the cell cycle we studied the labelling and appearance of histones in the nuclei during the early stages of the induction period. Figure 1b shows synthesis and appearance of histones and of total nuclear proteins in nuclei of *n*-butyrate treated cells. Radioactive labelling was done with ³H-arginine and ³H-lysine for 150 min in conditions where uptake of radioactivity was linear for more than 4 h. The rate of protein synthesis and rate of appearance of proteins in the nucleus steadily decreased during the 48-h period studied. This is similar to the early decrease in total cellular protein synthesis which has been recently reported for Friend cells in which haemoglobin synthesis has been induced by actinomycin D (ref. 14). In contrast to this decrease is the increasing level of newly synthesised histones in the nuclei: an initial period of 4 h with no change is followed by a pronounced increase in the amount of newly synthesised histones to a level two times greater than that in exponentially growing cells. This increase takes place concurrently with a decrease in the rate of DNA synthesis. These data suggest that an uncoupling of histone synthesis and DNA synthesis occurs before haemoglobin is produced. This dissociation represents an early effect after the addition of the inducer and leads to a higher rate of synthesis and transport of histones to the nucleus than in a state of exponential cell proliferation.

To establish whether all histone fractions respond in parallel or whether there is a differential effect in the different histone classes, labelled histones were separated by electrophoresis on SDS-polyacrylamide gels¹⁵. Histone bands located by Coomassie blue staining were excised and their radioactivity content determined. Separation of histones H3, H2a and H2b was not satisfactory and the three histones were treated as one fraction. The rate of accumulation of newly synthesised histones H1, H4 and (H3+H2a+H2b) is shown in Fig. 1c. The rate of appearance of the newly synthesised histones in nuclei is similar for all classes, indicating that the synthesis of nucleosomal histones and the synthesis of histones binding to DNA in the internucleosomal region are regulated coordinately and dissociated from DNA synthesis. This special mode of histone synthesis seems to occur in parallel with hyperacetylation of histones which has been recently described in *n*-butyrate-induced Friend cells¹⁶ and which seems to be due to inhibition of histone deacetylation¹⁷.

The changes in DNA and histone synthesis described here may reflect some intrinsic features of the commitment event. A prolonged G1 phase is characterised by an increase in the rate of appearance of newly synthesised histones in the nuclei. This increase is seen in all histone classes (including H1₀ (ref. 18) but not in total nuclear proteins which show a steady decrease in their rate of synthesis. Prolongation of the G1 phase may facilitate the synthesis and modification of regulatory molecules required for the S phase during which reprogramming towards the expression of the differentiated function may take place.

JORDANKA ZLATANOVA*

PETER SWETLY

Ernst Boehringer Institut für Arzneimittelforschung,
A-1121 Vienna, Austria

Received 3 August; accepted 26 September 1978.

* Present address: Institute for Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

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Cell specificity in metabolic activation of aflatoxin B₁ and benzo(a)pyrene to mutagens for mammalian cells

ENVIRONMENTAL chemical carcinogens are usually non-reactive and have to be enzymatically activated before they can manifest their biological effects^{1–6}. Many cell types, including those used for mutagenesis studies, are not capable of activating such procarcinogens; mutagenesis in these cells has been studied by the addition of an enzymatically active liver subcellular fraction^{7–9}. However, these liver subcellular fractions readily activate a wide spectrum of compounds into potent mutagens including those which are not carcinogenic to the liver. In addition subcellular preparations differ from intact cells in the profile of the metabolites^{10,11} and DNA adducts^{12,13} formed after metabolism of various potent carcinogens such as aflatoxin B₁ (AF), benzo(a)pyrene (BP), and 7,12-dimethylbenz(a)anthracene. These results suggest that the use of subcellular fractions does not truly simulate the *in vivo* situation and

Table 1 Binding of BP to the DNA of fibroblasts and liver cells

	BP binding (ng bound BP per mg DNA) after treatment with:	
	1.0 $\mu\text{g ml}^{-1}$	0.1 $\mu\text{g ml}^{-1}$
Fibroblasts	8.9 $\pm$ 3.6	2.0 $\pm$ 0.8
Liver cells	3.2 $\pm$ 0.4	1.1 $\pm$ 0.2

Binding of BP to cellular DNA was determined 24 h after treatment with BP of 10⁷ fibroblasts and 7 $\times$ 10⁶ liver cells grown in 10 ml of medium per 100-mm Petri dish; 5–7 Petri dishes were used per data point. For isolation of the DNA-BP products, the treated cells were trypsinised, washed extensively with phosphate-buffered saline (pH 7.4), and then lysed in buffer containing 10 mM Tris-EDTA, 100 mM NaCl, and 1% SDS (pH 7.4). Lysates were extracted twice with an equal volume of distilled phenol, once with an equal volume of 1 part phenol to 1 part chloroform-isoamyl alcohol (v/v 24:1), and a further three times with chloroform-isoamyl alcohol (24:1), resulting in a clear interface. The nucleic acids were precipitated from the aqueous phase with 2.5 volumes of absolute ethanol and centrifuged at 1,000g for 10 min at 4°C. The precipitate was dissolved in 0.1 SSC (0.015 M NaCl–0.0015 M sodium citrate), and the RNA was digested with ribonuclease A 50 U ml^{–1} for 40 min at 37°C. Phenol and chloroform extractions were repeated as described above, and the DNA was precipitated with 2.5 volumes of absolute ethanol in the presence of 0.1 M NaCl. The precipitate was dissolved in H₂O, and the DNA was determined quantitatively by absorbance at 260 μm . The ratio A₂₆₀ to A₂₈₀ was 1.95 $\pm$ 0.05. The BP bound to DNA was determined by dissolving an aliquot in scintillation solution and counting the samples in a Tri-Carb liquid scintillation spectrometer. Both ¹⁴C-labelled BP (62.3 mCi mmol^{–1}) and ³H-labelled BP (4.4 Ci mmol^{–1}) were used in the binding studies. Values means $\pm$ s.d. derived from four independent experiments, two with each type of label.

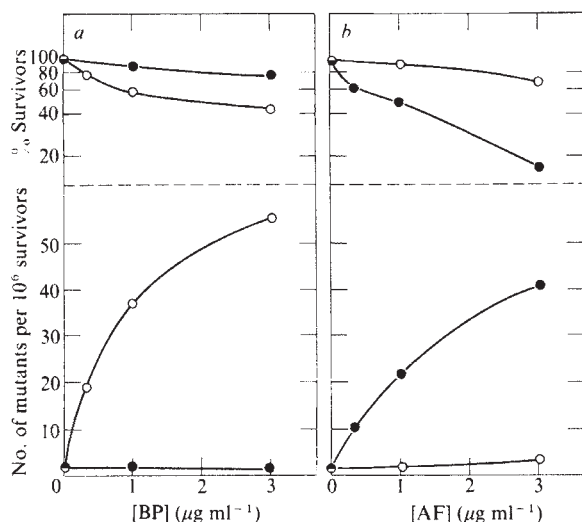


Fig. 1 Fibroblast- and liver cell-mediated mutagenesis by the carcinogens BP (a) and AF (b). Mutation to ouabain resistance was determined in Chinese hamster V79 cells after co-cultivation with either liver cells or fibroblasts derived from Sprague-Dawley rats. Liver cells were obtained after *in vivo* collagenase perfusion of a liver from a 6- to 10-week-old male rat^{18,23}. V79 cells were seeded at 10^5 cells per 25-cm² T-flask in 4 ml Williams medium containing 10% fetal calf serum supplemented with 2 mM glutamine, penicillin (100 U ml^{-1}), and streptomycin ($100 \mu\text{g ml}^{-1}$) at 37°C with an atmosphere of 5% CO_2 in air. After 18 h either 2×10^6 rat embryo fibroblasts (lethally irradiated with 5,000 R) or 10^7 primary rat hepatocytes (80–90% viable by Trypan blue dye exclusion and plating efficiency of 20%) were seeded on the V79 cells. After 3 h the medium was changed and the carcinogens were added in 8 ml of fresh medium. The cells were dispersed 48 h later with 0.05% trypsin and 0.02% EDTA, and the V79 cells, which could be distinguished in the haemocytometer from the fibroblasts and liver cells, were seeded at 200 cells per 50-mm Petri dish in 4 ml medium to determine cloning efficiency and at 10^5 cells per 50-mm Petri dish to determine the number of ouabain-resistant colonies. Ouabain was added 48 h later in 1 ml medium to a final concentration of 1 mM. Six dishes were stained with Giemsa 6–7 d after cell seeding to determine cloning efficiency, and 20 dishes were stained after 14–16 d to determine the number of ouabain-resistant colonies. Data are expressed as the number of ouabain-resistant mutants per 10^6 survivors based on the number of cells seeded for mutant selection and on the cloning efficiency. Data are the results of three separate experiments, and each data point was determined in duplicate. The number of mutants after induction with BP in the fibroblast-mediated assay and AF in the liver-cell-mediated assay varied up to about 25% in the three independent experiments. In all other cases the number of mutants in different experiments varied up to 40%. The cloning efficiency of V79 cells co-cultivated with liver or fibroblast cells was approximately 60%. Cytotoxicity is expressed as % survivors based on a comparison between the cloning efficiency of V79 cells co-cultivated in the absence of carcinogen and that in the presence of the carcinogen. After co-cultivation the number of V79 cells per T flask ranged from 1.2 to 2.8×10^6 cells. The heritability of ouabain resistance was shown with 10 isolated ouabain-resistant colonies, all of which remained resistant to the drug after 1 month in its absence. ●, Liver cells; ○, fibroblasts.

that it may be advantageous to use intact cells for metabolic activation of chemical carcinogens. Therefore, studies of carcinogen metabolism and mutagenic activity in cultured cells derived from different tissues should more truly reflect the *in vivo* situation and should also provide insight into the problem of tissue specificity in chemical carcinogenesis. Metabolic activation by intact cells of different carcinogens into intermediates which are mutagenic to mammalian cells has been reported^{4,14–19}. This report compares the abilities of two different cell types, fibroblasts and liver cells derived from rats,

to metabolise two carcinogens (BP, a potent skin and lung carcinogen which can also produce fibrosarcomas²⁰, and AF, a potent liver carcinogen²¹) to water-soluble products, to intermediates which bind to cellular DNA, and to intermediates which are mutagenic to Chinese hamster V79 cells. Ouabain resistance was used as the genetic marker in V79 cells^{14,22}.

Direct mutagenicity (that not requiring further metabolism of the carcinogens) was determined by testing the induction of ouabain resistance by AF and BP in V79 cells which do not metabolise these carcinogens. The results indicate that neither BP nor AF induced ouabain resistance in V79 cells at concentrations up to $3 \mu\text{g ml}^{-1}$. Co-cultivation of the V79 cells with rat fibroblasts which are capable of metabolising BP (fibroblast-mediated assay) or with rat liver cells which metabolise AF (hepatocyte-mediated assay) in the absence of the carcinogens did not alter the mutation frequency. BP in the fibroblast-mediated assay caused 17-, 34-, and 55-fold higher mutation frequencies than the control at 0.3, 1.0 and $3.0 \mu\text{g ml}^{-1}$, respectively (Fig. 1a), while in the hepatocyte-mediated assay no significant increase was observed in the mutation frequencies at BP doses up to $3 \mu\text{g ml}^{-1}$. Treatment of the V79 cells with AF at 0.3, 1.0 or $3.0 \mu\text{g ml}^{-1}$ in the hepatocyte-mediated assay resulted in 10-, 20- and 40-fold higher mutation frequencies than the control (Fig. 1b). In the fibroblast-mediated assay AF caused only a twofold increase at a dose as high as $3 \mu\text{g ml}^{-1}$.

The differences observed in the mutagenic activities of BP and AF in the hepatocyte- and fibroblast-mediated assays suggest that these two carcinogens are metabolised differently in each of the two cell types. Therefore the extent of BP and AF metabolism to water-soluble metabolites^{11,24} by hepatocytes and fibroblasts was compared. The results indicate that liver cells metabolise both BP and AF to water-soluble products, while fibroblasts metabolise BP but not AF (Figs 2, 3). For both cell types the metabolism of BP to water-soluble products was proportional to the cell number (Fig. 2a), the dose of the hydrocarbon (Fig. 2c), and the time of incubation up to 48 h (Fig. 2b). After 48 h, BP metabolism by liver cells reached a plateau, probably due to cell degeneration, while in the fibroblasts BP metabolism continued for an additional 24 h. The metabolism of AF by liver cells was also proportional to the cell number, the dose of the carcinogen, and the time up to 24 h (Fig. 2a–c). The liver cells showed only a limited increase in AF metabolism after 24 h, presumably due to the cytotoxic effects of AF metabolites and degeneration of the hepatocytes in culture. The fibroblasts did not metabolise AF (Fig. 3a–c), and this can explain the absence of AF mutagenicity in the fibroblast-mediated assay.

Therefore, even though on a per-cell basis, liver cells were able to metabolise BP to water-soluble products four to five times as effectively as fibroblasts (Fig. 2a–c), in the hepatocyte-mediated assay BP was not mutagenic (Fig. 1a). These findings raised the question of production and/or release of mutagenic BP metabolites by the liver cells. To estimate the relative production of reactive BP intermediates by the two cell types, the binding of BP metabolites to DNA of both cell types was measured. After 24 h exposure to BP at $1 \mu\text{g ml}^{-1}$, approximately three times as much BP was bound to the DNA of the fibroblasts as was bound to the DNA of the liver cells (Table 1). Thus even though the liver cells metabolised four to five times as much BP at $1 \mu\text{g ml}^{-1}$ to water-soluble products, they had substantially less DNA-bound BP products than the fibroblasts. These findings indicate either that liver cells metabolise BP to less reactive intermediates as compared with fibroblasts, or that if such reactive metabolites are formed they are rapidly detoxified. It has been reported that the degree of covalent binding of BP metabolites to DNA is regulated by their relative rate of production and their rate of detoxification²⁵.

The mutagenic activity of BP in the fibroblast-mediated assay but not in the hepatocyte-mediated assay and the inverse situation with AF are in agreement with the *in vivo* activities of these two carcinogens. However, 'S-9' subcellular fractions from liver tissue have been reported to activate both carcinogens to

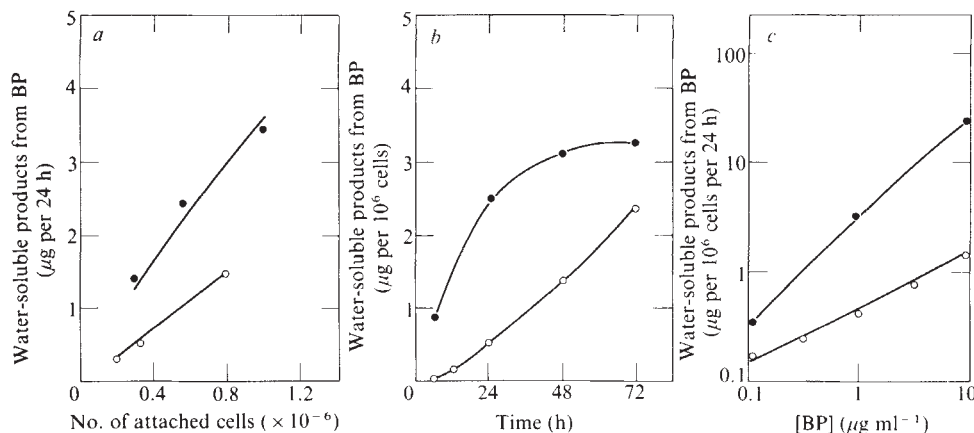


Fig. 2 Metabolism of BP by liver cells and fibroblasts. Liver cells were isolated after *in vivo* collagenase perfusion of the liver of a 6- to 10-week-old male rat, and the cells (viability, 80-90%) were seeded into 25-cm² T flasks in 5 ml medium. The plating efficiency was about 20%. Lethally irradiated (5,000 R) rat embryo fibroblasts were seeded in 5 ml of medium into 25-cm² T flasks. The plating efficiency of the fibroblasts was about 80%. At 3 h after seeding the medium was changed and 5 ml medium containing the ¹⁴C-AF or ¹⁴C-BP was added. The reaction was terminated by addition of 5 ml of a 1% SDS solution to each T flask²⁴.

The contents of the flask were then mixed with a pipette to ensure that all cells lysed, and 5 ml was removed and mixed with nine volumes of chloroform-methanol (2:1). The solution was vigorously mixed and then centrifuged. Duplicate 0.5-ml aliquots of the aqueous (upper) phase were removed and added to 10 ml Aquasol for counting in a Beckman Model B scintillation counter. Total recoveries were 90-100% as determined by summing radioactivities in the aqueous and organic phases. In the absence of cells, 0.3-0.6% of the ¹⁴C-AF were soluble in the aqueous phase; this was considered as background. All experiments were run with three dishes per point, and each experiment was repeated 2-3 times. The data presented are the mean values, and the variance between different experiments was up to 15%. Liver cell numbers were determined by counting five different fields of the T flask with a grid. Stock solution of the medium containing a known amount of the radioactive carcinogen AF ($2-3 \times 10^4$ c.p.m. ml⁻¹; [G-¹⁴C]AF) or BP ($4-5 \times 10^4$ c.p.m. ml⁻¹; [7,10-¹⁴C]BP) with the desired quantities of unlabelled BP or AF was added to each culture. Thus the total radioactivity in an individual experiment remained constant, but the specific activity decreased as the dose increased. ●, Liver cells; ○, fibroblasts.

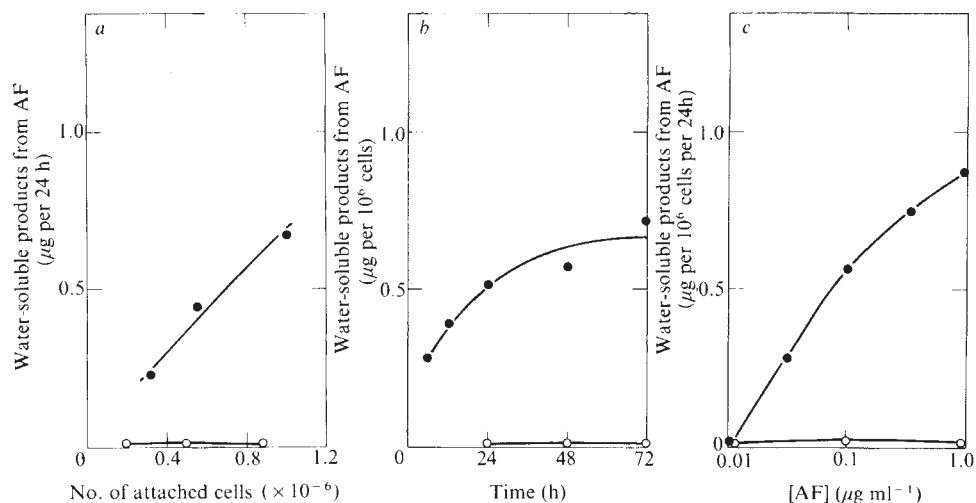


Fig. 3 Metabolism of AF by liver cells and fibroblasts. For details see Fig. 2. ●, Liver cells; ○, fibroblasts.

intermediates which are mutagenic for both *Salmonella typhimurium*⁷ and V79 cells⁸. These differences in the metabolic activation may result from the disruption in the liver subcellular preparations of the normal balance of pathways by which BP and AF are activated and detoxified in the intact liver.

Our results indicate that a cell type specificity in chemical carcinogenesis can be investigated by the cell-mediated assay. Furthermore, while the metabolism of procarcinogens is required to manifest *in vitro* mutagenic activity, overall metabolism alone is not sufficient to predict the activity of a carcinogen in a given cell type. The discrepancy between the metabolic activation of the same chemical carcinogens by subcellular fractions as opposed to intact cells and the loss of cell specificity by the former lead us to suggest that the cell-mediated assay may provide a more useful means of screening environmental chemicals and may also be used to study tissue specificity of chemical carcinogens *in vitro*.

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ROBERT LANGENBACH

*Eppley Institute for Cancer Research,
and
Biology Division, Oak Ridge National Laboratory*

HEATHER J. FREED

*Eppley Institute for Cancer Research,
University of Nebraska Medical Center,
Omaha, Nebraska 68105*

DINA RAVEH

*University of Tennessee-Oak Ridge
Graduate School of Biomedical Sciences,
and
Biology Division, Oak Ridge National Laboratory*

ELIEZER HUBERMAN

*Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830*

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Volatile nitrosamines in normal human faeces

NITROSAMINES are a major class of chemical carcinogens which lead to a wide variety of tumours in many animals¹. They may be formed *in vitro* by nitrosation of primary², secondary³ or tertiary amines⁴ and are found in air⁵, water⁶, foods⁷ and consumer products⁸. They may also be formed *in vivo* in the stomach or small intestine of experimental animals following the concurrent administration of nitrite and precursor amines^{9–12}. Here we show that several volatile nitrosamines are present in normal human faeces and suggest that they have been produced endogenously in the lower gastrointestinal tract.

Previously, we had used freeze-dried faecal samples for studies of the mutagenic compounds (presumptive carcinogens) in faeces¹³. However, this method does not test for volatile compounds of possible interest. Hence, the freeze-drier condensate was extracted with dichloromethane and this extract was injected on either a gas-liquid chromatograph

(GLC) or a high-pressure liquid chromatograph (HPLC), connected to a thermal energy analyser (TEA). Several peaks were observed (Fig. 1) and were attributed to nitroso compounds, as the TEA detector is highly specific for NO-containing compounds. By comparison with standards, the five major peaks were tentatively identified as due to *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosomorpholine (NMOR), the amounts present in a typical condensate being, respectively, 0.04, 0.10, 0.08, 0.08 and 0.02 µg per l.

The assignment of the TEA peak from the faecal condensate was confirmed by four methods. First, the estimates of the five nitrosamines obtained by either GLC or HPLC were found to agree quantitatively. Second, the isolated fraction corresponding to the peak for NDMA on HPLC was found to give only one peak on GLC-TEA, corresponding to the position of NDMA. The same was true for NDEA, NDBA, NPIP and NMOR.

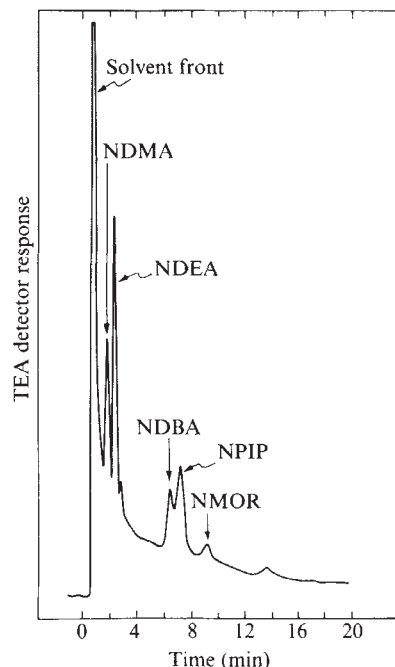


Fig. 1 GLC for a 5-µl DCM extract of the condensate from the freeze drier. 14 feet of stainless steel tubing (outer diameter 1/8th inch), packed with 10% Carbowax 20M on Chromosorb W, 80–100 mesh was used for a single-column isothermal gas chromatograph. The carrier gas was argon at a flow rate of 30 ml min⁻¹ and the column oven temperature was 150 °C. A model TEA-502 thermal energy analyser (TEA, Thermo Electron) was used as the detector. TEA attenuation was ×8. The last peak on the chromatogram is unidentified.

Table 1 Volatile nitrosamines in normal human faeces

Donor	Men (µg per kg)*				
	NDMA	NDEA	NDBA	NPYR	NMOR
A	0.94	6.76	0.87	0.31	0.23
B	0.27	1.76	0.12	ND	0.07
C	0.51	11.32	ND	ND	0.45
D	0.22	1.91	ND	ND	ND
E	1.20	2.16	ND	ND	1.25
F	0.27	0.18	ND	ND	ND
G	1.11	ND	ND	ND	ND
Donor	Women (µg per kg)*				
	NDMA	NDEA	NDBA	NPYR	NMOR
H	1.47	2.81	ND	0.78	ND
I	1.06	ND	ND	ND	ND
J	1.49	13.3	ND	0.55	0.51
K	ND	ND	ND	ND	ND
L	0.63	ND	ND	ND	ND

ND, not detectable (<0.01 µg per kg); NDMA, nitrosodimethylamine; NDEA, nitrosodiethylamine; NDBA, nitrosodibutylamine; NPYR, nitrosopyrrolidine; NMOR, nitrosomorpholine.

* After correction with recovery efficiency as shown in text.

Third, when the dichloromethane extract of the condensate was treated with HBr in glacial acetic acid it no longer gave any peaks on the TEA detector. The same was true when the extract was transferred into aqueous acid solution and exposed to ultraviolet light. As both these treatments are known to cleave the N–NO bond of nitrosamines^{14,15}, the peaks seen in Fig. 1 were presumably due to nitrosamines. Fourth, the fractions corresponding to NDBA and NPIP were purified by HPLC after several clean-up procedures¹⁶ and the presence of the compounds in the purified fractions was confirmed by comparing the spectra obtained with mass spectroscopy (GLC-MS) with that obtained with standards for NDBA and NPIP¹⁷. The finding that faecal distillates contained volatile nitrosamines prompted an extensive investigation of these compounds in normal faeces.

Faecal samples were collected from 12 healthy volunteers at the Ontario Cancer Institute and immediately extracted with

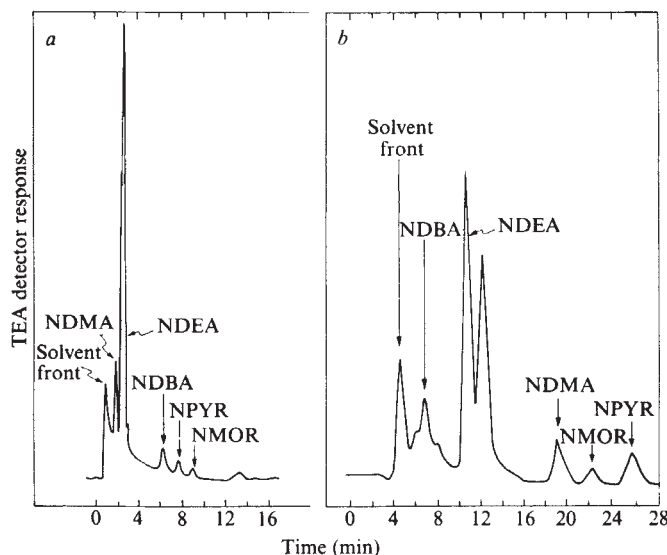


Fig. 2 *a*, GLC for a 10- μ l DCM extract of the faecal sample from donor A. The operating conditions for GLC-TEA were identical to those described in Fig. 1. *b*, HPLC for a 20- μ l DCM extract of the faecal sample from donor A. The HPLC-TEA was constructed by connecting a model UK6 injector (Waters Associates) with a model 6000A high-pressure pump solvent delivery system (Waters Associates) and a model TEA-502 thermal energy analyser (Thermo Electron). A μ -porasil column (0.39 cm inner diameter $\times$ 30 cm; Waters Associates) was used with a 5% acetone-95% 2, 3, 4-trimethylpentane solvent system at a flow rate of 1 ml min⁻¹. TEA attenuation was $\times 8$. The peak after NDEA on HPLC chromatogram is unidentified.

dichloromethane. The extract was dried with sodium sulphate, concentrated and then distilled from mineral oil to a liquid nitrogen cold trap¹⁸. The distillate was extracted with dichloromethane and then applied to GLC-TEA or HPLC-TEA in conditions similar to those described by Fine¹⁹. Control experiments showed that the reagents and chemicals used in the analytical procedure contained no volatile nitrosamines and that the addition of ascorbic acid to the faeces, at a concentration which inhibits nitrosation of amines²⁰ (1 mg per g of faeces), did not affect the yield. With this method, the recovery of NDMA, NDEA, NDBA, NPIP, NPYR and NMOR at a concentration of 2 μ g per kg added to the faeces was found to be 51, 68, 80, 81, 88 and 88%, respectively.

Results of a typical GLC-TEA and HPLC-TEA tracings for the faeces from one volunteer (donor A) are shown in Fig. 2. The identification of the peaks as NDMA, NDEA, NDBA, NPYR and NMOR in Fig. 2 was confirmed by the first three methods described above. The results for 12 samples (Table 1) show that most faeces contain NDMA at levels of 0.3–1.5 μ g per kg. NDEA was found in higher amounts (0.2–13 μ g per kg), with samples from both males and females giving values >10 μ g per kg. Two samples had detectable levels of NDBA, in both cases <1 μ g per kg. NPYR and NMOR were found in several donors, with detectable levels <2 μ g per kg. Many of the levels here are higher than those found in the freeze-dried condensate. Presumably, losses occurred during the freeze-drying period and not all the volatile nitrosamines were retained in the freeze drier.

The source of the nitroso compounds in the faeces has not been established. Perhaps some may arise in food or from nitrosation in the stomach but it seems more likely that they are produced in the lower gastrointestinal tract, as Tannenbaum *et al.*²¹ have shown that the nitrite precursor may be present at these sites. It has been suggested that the intestine might be a site for the formation of nitrosamines by bacterial action²². The formation of nitrosamines from precursor amines and nitrate at neutral pH by some strains of bacteria has been demonstrated by Sander²³ and the formation of *N*-nitrosodimethylamine on

incubation of ¹⁴C-dimethylamine and sodium nitrite with rat faecal contents has also been reported^{24,25}. The levels of volatile nitroso compounds in human faeces are similar to those in some foods⁷. However, the long, continuous exposure to nitroso compounds in faeces could pose a greater hazard than that from many foods. The volatile nitroso compounds present in the faeces will presumably be absorbed and distributed throughout the body. Indeed, the absorption from this site may explain the presence of nitroso compounds in the blood¹⁹ and urine²⁶ of normal individuals. Although the presence of these nitroso compounds in the human body is surprising, it remains to be determined whether they are responsible for any human cancers.

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T. WANG
T. KAKIZOE
P. DION
R. FURRER
A. J. VARGHESE
W. R. BRUCE

Ontario Cancer Institute,
500 Sherbourne Street,
Toronto, Ontario,
Canada M4X 1K9

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Tetrahydropapaveroline induces small granular vesicles in brain dopamine fibres

TETRAHYDROISOQUINOLINES (TIQs) can be formed by the condensation of catecholamines with aldehydes. Their *in vivo* formation has been postulated as a possible mechanism to explain some long-term effects of ethanol on the central nervous

system^{1,2}. However, there have been several criticisms^{3,4} and reviews^{5,6} of this hypothesis. Although TIQ formation may not explain all the effects of ethanol, TIQs can influence behaviour⁷⁻⁹ and they do have transmitter-like properties: they can be formed *in vivo*¹⁰⁻¹²; they can be taken up and released *in vivo* from the adrenergic plexus of the rat iris¹³; they can be taken up into rat brain synaptosome fractions¹⁴; and TIQs applied exogenously have been localised in peripheral catecholamine tissues with both fluorescence¹⁵ and electron microscopy¹⁵. Thus, TIQs may accumulate in specific brain regions and influence behaviour by either acting as false transmitters¹³ or by displacing endogenous transmitters. Critical to this TIQ hypothesis is the demonstration of TIQ uptake and localisation into central neurone terminals. In the present study, slices of rat caudate nucleus were incubated with TIQs and then examined with the electron microscope. The results provide evidence for both the uptake and localisation of the TIQ, tetrahydropapaveroline (THP), into central dopamine fibres and terminals. This localisation may prove important in understanding the mechanism by which TIQs influence animal behaviour and any possible role for TIQs in chronic alcohol consumption.

We used male Sprague-Dawley rats (250–350 g); razor-blade slices of caudate nucleus were incubated for 30 min at 37 °C with various compounds at 5×10^{-4} M (refs 16–18). Tissues were then fixed with potassium permanganate and examined under the electron microscope¹⁹. After incubation with THP, small granular vesicles (SGVs) were seen in 5–6% of the bouton population (Fig. 1 and Table 1). These SGV boutons were similar in appearance to those identified by others as containing dopamine^{16-18,20}.

Two further experiments were carried out to determine whether the THP-induced SGV boutons were dopamine terminals. First, as dopamine is actively accumulated^{21,22} but does not normally produce a granular precipitate within dopamine-containing synaptic vesicles^{16,23}, it might block THP uptake and thus the appearance of SGVs in dopamine terminals. Table 1 shows that caudate nucleus incubated with both THP and a 10-fold concentration of dopamine (5×10^{-3} M) was practically devoid of THP-induced SGV boutons. Second, as striatal dopamine innervation is predominantly ipsilateral^{20,24}, a unilateral lesion of its source nucleus, the substantia nigra,

Table 1 Tetrahydropapaveroline-induced small granular vesicle-containing boutons (THP-SGV boutons) in the rat caudate nucleus

	THP-SGV boutons	Total boutons	%THP-SGV boutons
Caudate nucleus from non-lesioned rats			
THP only	497	8,806	5.6
THP + dopamine (5×10^{-3} M)	2	10,391	0.02*
Caudate nucleus after unilateral substantia nigra lesion			
Contralateral to lesion	629	11,761	5.3
Ipsilateral to lesion	48	12,141	0.4*

Slices of rat caudate nucleus were incubated with 5×10^{-4} M THP as outlined in the text. Three unit areas of $6,500 \mu\text{m}^2$ from each rat (two rats in each group) were examined.

* $P < 0.001$ (χ^2 test).

should cause a decrease in dopamine terminals on the lesioned side^{20,24}. Table 1 shows that after unilateral 6-hydroxydopamine lesions^{20,25} <1% of the boutons contained THP-induced SGVs as compared to 5–6% in the non-lesioned contralateral hemisphere. Substantia nigra lesions were confirmed by both a decreased glyoxylic acid-induced monoamine fluorescence (ref. 26, without perfusion) in the ipsilateral caudate as compared to the non-lesioned contralateral side and a pronounced contralateral asymmetry 10–15 min after a subcutaneous injection of apomorphine (0.25 mg per kg). Although these results are not conclusive, they indicate that THP can be accumulated in central nerve terminals, that these terminals contain dopamine and that they arise from cell bodies in the substantia nigra. Other monoamine terminal regions might also be expected to accumulate THP.

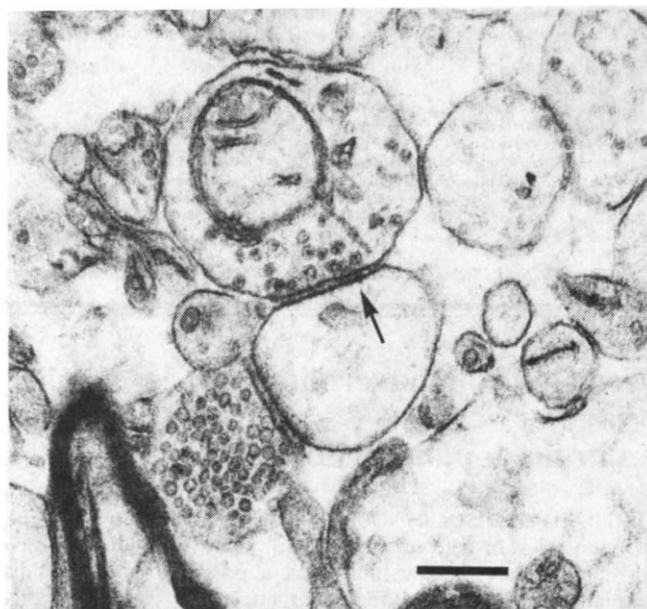
Our object was in part to identify the THP-induced SGV boutons as either dopamine- or non-dopamine-containing terminals. Thus, the drug concentrations used (5×10^{-4} M) were well above selective uptake levels but were similar to the concentrations used by others to demonstrate dopamine terminals^{17,18}. In addition, lower concentrations of THP reveal fewer dopamine terminals²⁷. Interestingly, incubation with another dopamine-derived TIQ, salsolinol, and also tetrahydropapaverine, apomorphine and morphine (all at 5×10^{-4} M) failed to produce SGV boutons. Incubation with α -methylnoradrenaline or 5-hydroxydopamine (5×10^{-4} M) revealed SGVs in 10–11% of the striatal bouton population; this is similar to values reported by others^{18,20}.

Compounds localised within vesicles may or may not produce a granular precipitate after permanganate fixation. The efficiency with which a compound produces a precipitate may be determined by its reactivity towards the permanganate ion, or by its ability to precipitate with manganese dioxide²³. Thus, the presence of a granular precipitate within vesicles probably indicates uptake, but the absence of precipitate could simply reflect the inefficiency or inability of a compound to sequester the electron-opaque manganese dioxide.

Reports by Myers and co-workers⁷⁻⁹ have renewed interest in the possible relationship of the TIQs to the physiological processes involved in alcohol addiction. In particular, intraventricular injections of THP were reported to induce rats to consume excess quantities of alcohol voluntarily as compared to control rats⁷⁻⁹. It was also reported that another TIQ, salsolinol, and a β -carboline, noreleagine, caused rats to drink alcohol in excess of controls.

If TIQs are important mediators of the effects of ethanol on the brain, the central catecholamine terminals would provide an opportune target for TIQ formation, storage and release. The present study presents ultrastructural evidence that exogenous TIQ can be accumulated and stored in central dopamine terminals. This cytochemical strategy may prove useful in future efforts to prove or disprove the TIQ hypothesis of alcohol action.

Fig. 1 Electron micrograph. The arrow in the dendrite points towards a tetrahydropapaveroline-induced small granular vesicle-containing bouton in the rat caudate nucleus. Note that these granular vesicles are larger in diameter than the agranular vesicles seen in the adjacent bouton. Scale bar, 250 nm.



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L. Y. KODA
G. F. KOOB
W. T. SHIER
F. E. BLOOM

Arthur Vining Davis Center for Behavioral Neurobiology,
The Salk Institute,
La Jolla, California 92037

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Primary afferent depolarisation of tooth pulp afferents is not affected by naloxone

RECENT studies (for review see ref. 1) have indicated the existence of endogenous opiate-related peptides and opiate receptors in the dorsal horn of the spinal cord and in the trigeminal nucleus caudalis (the caudal-most component of the trigeminal spinal tract nucleus in the brain stem). Both structures have marginal and substantia gelatinosa layers, receive the terminals of Aδ and C nociceptive afferents, and constitute essential components of spinal or trigeminal nociceptive pathways^{2,3}. It has been suggested^{1,4-7} that endogenous opiate-related peptides located in presynaptic endings play an important part in nociceptive transmission by inhibiting the presynaptic input to neurones in nociceptive pathways; presynaptic inhibition is thought⁸ to result from primary afferent depolarisation (PAD). It has previously been found that jaw-opening reflex activity⁹⁻¹¹ and the responses of trigeminal brain stem neurones^{10,11} to noxious orofacial stimuli can be suppressed by stimulation of the periaqueductal grey matter (PGM) and that this descending control is at least partially narcotic-related in the cat, as it can often be reversed by administering the opiate antagonist naloxone. Furthermore, the iontophoretic application of the opiate-related peptide enkephalin also depresses the activity of these neurones^{12,13}. If the opiate-related peptides are in fact involved in this suppression at a presynaptic level, then by stimulating PGM and related structures one should be able to produce PAD in nociceptive afferents and then abolish the PAD by naloxone administration. We describe here the first test of this proposition, using the tooth pulp (TP) as the source of

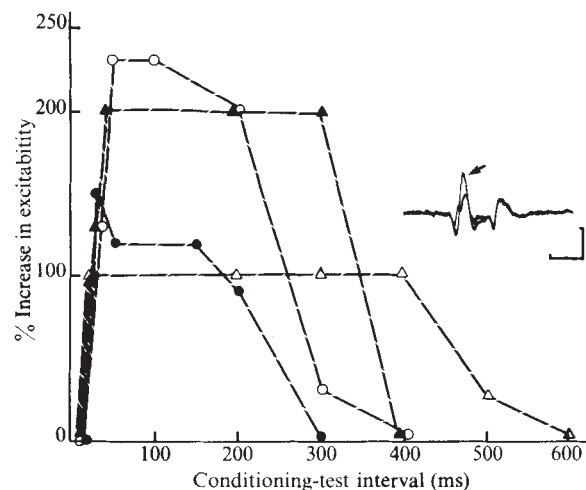


Fig. 1 Increase in antidromic excitability of a single mandibular canine tooth pulp (TP) afferent ending in trigeminal nucleus caudalis produced by conditioning stimulation of PGM (●), NRM (○), maxillary canine TP (▲) and mandibular skin (△). At each conditioning-test interval, the incidence of a spike evoked antidromically by 10 test stimuli to the caudalis was compared with that evoked by a similar number of caudalis stimuli in the presence of a conditioning stimulus. An increase in incidence produced by the conditioning stimulus was expressed as a % increase in antidromic excitability. The insert shows the spike (arrow) evoked antidromically at threshold by one caudalis stimulus, superimposed on a trace where the caudalis stimulus did not elicit the spike although other antidromic activity was evoked. Voltage calibration, 0.2 mV; time calibration, 2 ms.

nociceptive afferents^{14,15}. We have found that these afferents are subject to PAD which, however, cannot be abolished by naloxone.

Most of the methods used have been described in detail elsewhere^{10,16,17} and will only be briefly described. Thirty cats were anaesthetised with chloralose (60 mg per kg) or decerebrated. Blood pressure, rectal temperature and end-tidal % CO₂ were monitored continuously. In five of these cats, we recorded the activity of single primary afferent units in the trigeminal ganglion that could be orthodromically excited by bipolar stimulation of the ipsilateral canine TP¹⁷ and antidromically activated by brain stem stimulation. In most animals, however, bipolar recordings of TP afferents were actually made from the TP. For such recordings, two drill holes were placed in each of the canine teeth, to a depth at which only a very thin layer of dentine was left, and the TP was carefully exposed by inserting a sharp needle through the thin dentine wall. Bipolar platinum electrodes were implanted in each TP with dental cement. The brain stem was exposed and electrodes located stereotaxically in the trigeminal brain stem nuclei oralis and caudalis, using recordings of maximum field potentials evoked by TP or infra-orbital nerve (IO) stimuli, and histological verification. Usually, single-unit activity as well as compound action potentials could be antidromically evoked from TP by stimulation (0.1-ms pulse) of these brain stem nuclei. The rate of brain stem stimulation was limited to 0.5-1 Hz. We noted that at brain stem stimulation rates above 1 Hz, the amplitude of the antidromically evoked compound action potential was enhanced. This would reflect increased excitability and PAD, presumably due to a temporal summation effect induced by preceding brain stem stimuli.

Electrodes were also stereotaxically placed (with histological verification) in the following structures for subsequent conditioning stimulation: PGM, contralateral thalamic nucleus ventralis posteromedialis (VPM), nucleus raphe magnus (NRM) and adjacent reticular formation. Bipolar stimulation of these three sites, plus IO, TP and facial skin, were used as conditioning stimuli (10 ms, 400-Hz train, or 200 ms, 100-Hz train, or single 0.2-ms pulse). Mechanical stimuli applied to the facial skin were

also used, and included an alligator clamp described as noxious when applied to human skin, a brush and an electronically controlled mechanical probe. The presynaptic modulation by these various conditioning stimuli of TP afferents was determined by measuring a change in their antidromic excitability. An increase in amplitude of the antidromically evoked compound action potential or increase in the probability of antidromic activation of single TP units, were used as measures of PAD^{8,16,18}.

Table 1 Incidence of PAD produced in TP afferent endings in nucleus oralis or nucleus caudalis by various conditioning stimuli

Conditioning stimulus	Oralis	Caudalis
PGM	66% (47)	64% (64)
NRM	83% (18)	73% (37)
VPM	90% (10)	76% (25)
IO	81% (11)	68% (19)
TP	87% (14)	75% (20)

The numbers in parentheses indicate the number of units tested with each conditioning stimulus.

The following criteria were used to distinguish single-unit activity from compound action potentials: the clear and consistent shape of the former, displaying 'all-or-none' firing at its threshold for antidromic activation, with a constant latency and ability to follow high-frequency (100 Hz) stimulation (to exclude the trigeminal tract reflex¹⁹ which we observed occasionally). A total of 150 units were recorded in TP or the trigeminal ganglion. Antidromically induced compound action potentials and single-unit activity had latencies ranging over 2–6 ms, consistent with TP afferents conducting primarily in the A δ range^{14,20}.

All the conditioning stimuli used were capable of inducing PAD of TP afferent endings in oralis or caudalis as judged by changes in the antidromic excitability of compound action potentials and single units recorded either in the trigeminal ganglion or ipsilateral TP. There was no selective presynaptic modulation of the small-diameter (TP) afferents by large afferent fibre activation, and our findings of peripherally induced PAD are supported by an earlier report of PAD in TP afferents²⁰; however, centrally evoked PAD of TP afferents has not previously been reported (Table 1). Not every stimulation site was effective for every unit, even in the same animal and using the same conditioning and test sites. In general, the incidence of PAD was somewhat lower for endings in caudalis than for those in oralis (Table 1), in agreement with previous reports^{16,19} of PAD in trigeminal afferents. The time course of PAD in a TP unit is shown in Fig. 1. PAD was detected at conditioning-test intervals greater than 20–30 ms, was maximal at around 50 ms and lasted for 100–500 ms.

In 22 TP units showing PAD with PGM, NRM or high-intensity skin conditioning stimulation we also tested the effect of naloxone administration (0.4 mg per kg, intravenously); 14 of these 22 units were antidromically excited from caudalis, and eight from oralis. We chose these 22 units for testing because of their consistent and distinctive shape, and the PGM and NRM conditioning stimuli were set at their lowest intensity for producing a reliable PAD effect. Thus, conditions were made as favourable as possible for detecting the reversal of PAD with the opiate antagonist naloxone that would be expected if the presynaptic excitability change induced by these stimuli is related to the action of endogenous opiate-related peptides. The PAD effect was monitored over a period of 25 min after the initial intravenous administration of the drug. Naloxone did not produce any consistent change in baseline antidromic excitability of these units, and only in one of the 22 units tested was there any evidence of a naloxone-induced reduction in PAD. In

this unit, the PAD was produced in its oralis endings (antidromic latency 2.3 ms) by both PGM and NRM conditioning stimuli. However, this reduction of PAD as a result of naloxone administration could be overcome by a small increase (90–140 μ A) in conditioning stimulus intensity. The effect of naloxone lasted for 15 min, but recurred when a second dose of naloxone (0.4 mg per kg) was given 28 min after the initial administration. No reduction in PAD was noted, however, after naloxone administration in any of the other 21 units; seven of the units were tested with a second dose of naloxone (0.4–2.0 mg per kg, 15–30 min after initial dose), but again no reversal of PAD was noted.

In conclusion, the various conditioning stimuli used were capable of producing PAD in TP afferents. Stimuli to PGM and NRM, sites implicated in several studies^{2,21,22} as sources of descending influences related to narcotic analgesia, were very effective in producing PAD. This would suggest that the PAD produced from these sites is related to narcotic analgesia. The opiate antagonist naloxone, however, failed to reverse PAD produced by PGM or NRM (or high-intensity facial) stimulation in 21 of 22 units tested. This may indicate that opiate receptors and opiate-related peptides are not involved in presynaptic modulation of nociceptive input from primary afferents^{1,4–7}. However, there are alternative explanations. Our sample of TP units was probably biased towards the large-diameter TP afferents, and although unlikely, it is nevertheless conceivable that the TP units we tested with naloxone may have been involved in functions other than nociception¹⁵. In addition, although PGM and NRM constitute likely candidates for sites producing modulation, the actual origin of the hypothetical enkephalin terminals on the putative presynaptic opiate receptors is uncertain^{2,21,22}. Thus, it is possible that our conditioning stimuli may not have activated a presynaptic enkephalinergic pathway. It is also possible that opiate receptors mediate presynaptic inhibition by some process other than PAD⁸.

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JAMES W. HU

JONATHAN O. DOSTROVSKY

BARRY J. SESSLE

*Division of Biological Sciences,
Faculty of Dentistry and Department of Physiology,
University of Toronto,
Toronto, Canada, M5G 1G6*

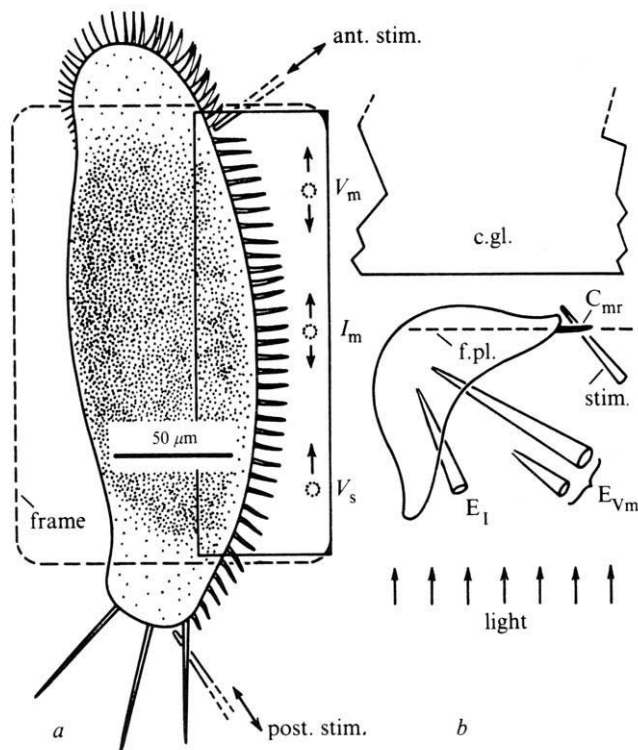
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Are receptor-activated ciliary motor responses mediated through voltage or current?

THE locomotion of ciliate protozoa is effected by cilia which modify the direction and frequency of their cyclic beating in response to sensory input from the environment^{1,2}. In *Paramecium* and *Stylonychia* mechanical stimulation of the anterior end of the cell induces a Ca^{2+} -dependent depolarising receptor potential which triggers a regenerative Ca^{2+} response^{3,4}. Ca^{2+} accumulating in the ciliary space activates the axonemal motor system⁵ so that the normal (posterior) direction of the ciliary power stroke is reversed and the frequency is augmented⁶. Posterior mechanical stimulation of the cell elicits a K^{+} -dependent hyperpolarising receptor potential followed by augmentation of ciliary frequency without reversal^{4,7}, a mechanism which is less well understood⁸. Because shifts in voltage are associated with loops of electric currents across the membrane, we have examined the crucial signals transmitted from the mechanoreceptive regions to the cilia. We report here that these signals are potentials of either polarity. This is the first report of intracellularly recorded receptor currents.

Fig. 1 *Stylonychia mytilus*, as orientated in the experimental set-up for cinematographic (16 mm, 250 f.p.s.) and electrophysiological recording. *a*, Cell in oblique dorsal view with right marginal cirri (C_{mr}) focused under a compound microscope (Zeiss interference contrast). Vertical oscilloscope deflections of membrane potential (V_m), membrane current (I_m) and driving voltage of piezoelectric stimulator (V_s) optically superimposed on frame. Positions of glass rod for anterior and posterior mechanical stimulation (ant. stim., post. stim.) are indicated. The rectangle marks the outline of photographs in Figs 2–4. Bar, 50 μm . *b*, Position of cell under a cover glass (c.g.l.) in the experimental chamber. Electrodes for current passing (E_i) and recording of the membrane voltage (E_v) were bent to be inserted from below. The position of the glass rod (stim.) was approximately 3 μm from the cell margin before a mechanical stimulus. Experiments were carried out at 18°C.



Single specimens of the freshwater ciliate *Stylonychia mytilus* were mounted on glass microelectrodes (1 M KCl, 40–60 M Ω) under a compound microscope in a solution of 1 mM CaCl_2 , 1 mM KCl and 1 mM Tris-HCl at pH 7.3, to register the stimulus-dependent electrical events as well as the movements of the cirri (Fig. 1). The marginal cirri (10–20 individual cilia, 15–20 μm long) are largely inactivated during the resting state of the cell which occurs while *Stylonychia* 'sits' on substrate surfaces, whereas other compound cilia (membranellae) beat rapidly to produce a feeding water current. Activation of the marginal and ventral cirri produces forward and backward

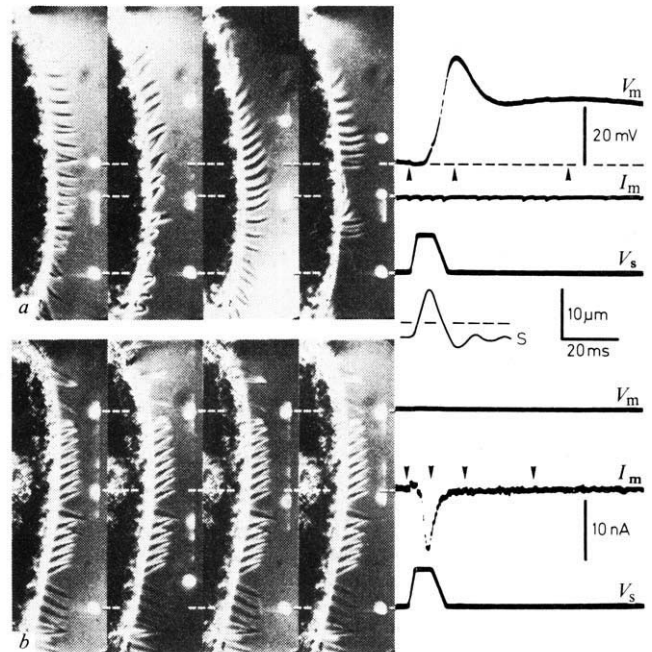


Fig. 2 Electrical and ciliary motor responses to mechanical stimulation of the anterior end of the cell. *a*, Sequence of frames (from left to right) to illustrate the onset of beating of the cirri towards the cell anterior following application of the mechanical stimulus. Oscilloscope traces (right) are graphically joined to signals on photographs (left). Arrowheads mark occurrence of represented frames (4th frame, 140 ms after start of stimulus). Actual tip excursion of needle (S) is indicated below trace of driving voltage (V_s); dashed line limits dead space between resting needle and cell surface⁴. Note activation of cirri during the rising slope of the action potential which rides on top of a receptor depolarisation. Artifacts on current trace (I_m) were picked up from flashes for film exposure. *b*, Absence of motor responses of the cirri after the same mechanical stimulus during voltage clamp of the resting potential. Current trace shows inward receptor current. Conventional oscilloscope display in *b* was recorded directly after filmed sequence to remove amplified flash artifacts from the current trace.

'walking' locomotion⁹. The experimental cells assumed a steady resting stage 2 min after fixation by two intracellular electrodes (Figs 2–4, leftmost frames) with a resting potential of -51 ± 3 mV.

Mechanical stimuli applied to the anterior membrane by the tip of a fine glass rod induced a ciliary motor response within 6–9 ms, coincident with the rising slope of a membrane depolarisation. This electrical response had an early receptor component followed by an action potential¹⁰ (Fig. 2*a*). The activated cirri swung anteriorly to perform several vigorous beating cycles (up to 60 Hz) with the power strokes orientated anteriorly. There was no activation of the cirri when the cell was similarly stimulated while the membrane voltage was clamped to the resting potential by negative feedback to remove the voltage signal (Fig. 2*b*). In this case the anterior mechanical stimulus produced an inward membrane current which rose to a peak (up to 10 nA) within 5 ms and decayed with a time constant of 3 ms, a fraction of the time constant of the decaying depolarising

receptor potential reported elsewhere⁴. The result suggests that a depolarising receptor potential from a brief conductance increase of the anterior receptor channel passively invades the cilia¹¹ to activate the voltage-sensitive channel of the ciliary membrane^{12,13}.

Mechanical stimulation of the cell posterior elicited a ciliary motor response (up to 45 Hz) within 10–13 ms together with a hyperpolarising receptor potential (Fig. 3a, 11 ms risetime to 26 mV amplitude, 35 ms time constant of decay). In contrast to the effects of depolarising stimuli, the first (Fig. 3a, 2nd frame from left) and subsequent ciliary power strokes were directed towards the posterior end of the cell. On the other hand, on stimulation with the membrane potential clamped to its resting value, the cirri remained unaffected, while a strong outward receptor current (Fig. 3b, 10 nA, 5 ms risetime, 7 ms time constant of decay) was recorded. The data shown in Figs 2 and 3 suggest that the ciliary apparatus can specifically respond to receptor-dependent depolarisation or hyperpolarisation, but not to the ionic currents accompanying receptor-site activation.

Motor effects of three types of inward membrane currents of similar amplitudes were examined to test the latter hypothesis.

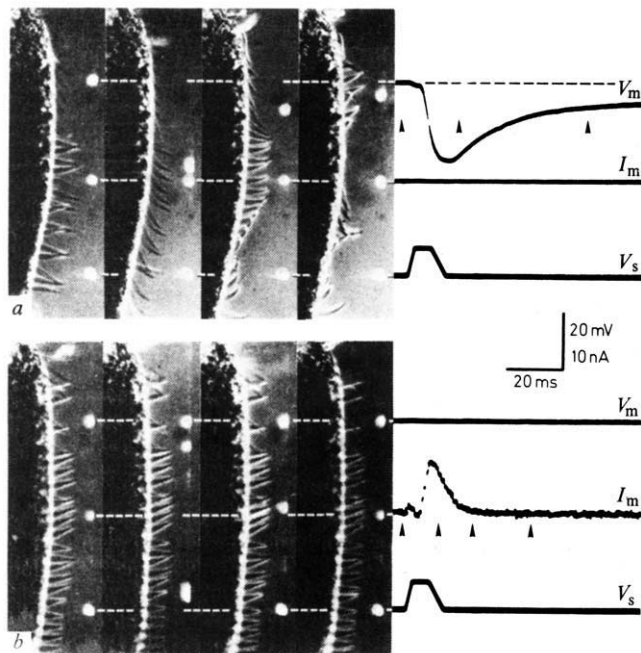


Fig. 3 Effects of posterior mechanical stimulation. *a*, Resting cirri (frame at left) start beating towards the rear of the cell after the occurrence of a hyperpolarising receptor potential (4th frame, 236 ms after stimulus onset). *b*, With the membrane voltage clamped at the resting potential, the same mechanical stimulus gives rise to an outward receptor current (I_m), but the compound cilia remain in the pre-stimulus resting position.

Although an inward receptor current could not activate the cirri in the absence of membrane voltage displacement (Fig. 2b), the early inward current due to a depolarising voltage step was accompanied by a reversal response of the cirri (Fig. 4a). Inward current from a hyperpolarising voltage step occurred in conjunction with ciliary activation towards the posterior of the cell (Fig. 4b). Comparison of Figs 2–4 unequivocally confirms signal transmission to the cilia by means of changes in voltage, not membrane currents. The failure of an inward receptor current to activate the cilia is interesting, as this current is carried by calcium. Estimates of the total ciliary fluid space and the somatic volume ($3 \times 10^{-9} \text{ cm}^3$, $4 \times 10^{-7} \text{ cm}^3$; H.M., unpublished), show that a receptor current of 10 nA restricted to the cilia increases the intraciliary Ca^{2+} concentration beyond 10^{-4} M , and raises the internal concentration by $3 \times 10^{-6} \text{ M}$, if Ca^{2+} is evenly distributed in the total cellular space. Both concentrations

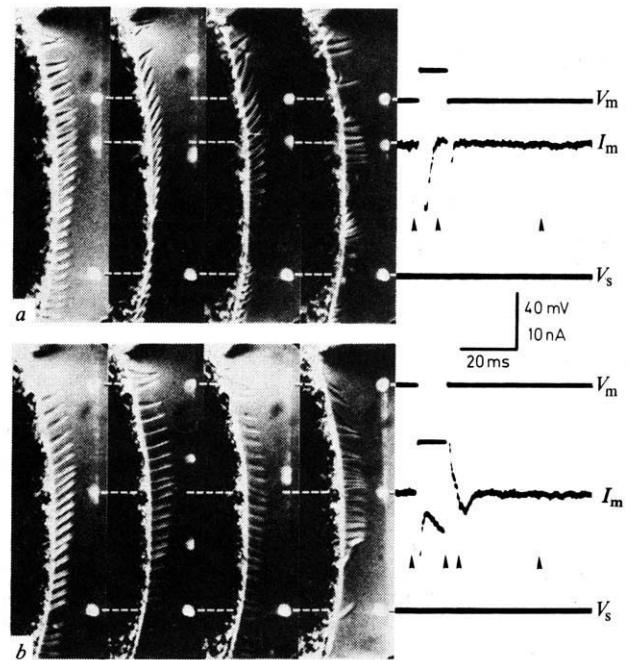


Fig. 4 A test of the adequacy of electrical signal for ciliary activation. *a*, A depolarising voltage step (V_m) produces an early inward current (I_m) and causes the cirri to beat towards the cell anterior (4th frame, 158 ms after voltage stimulus). *b*, A hyperpolarising voltage step accompanied by 'late inward' current activates cyclic beating of the cirri towards the posterior end of the cell. Note that the polarity of the voltage signal determines the ciliary response; the polarity and the size of the membrane current, which is similar to that in Fig. 2b (no ciliary activation), is not a signal recognised by the cilia.

are sufficient for ciliary reversal^{5,14}. Thus, we suggest that Ca^{2+} -receptor channels in *Stylonychia* occur exclusively on non-ciliary membranes. A K^+ -dependent hyperpolarising mechanoreceptor channel was also localised on the somatic surface in deciliated *Paramecium*¹³, whereas membrane mutants of *P. caudatum* which fail to produce membrane electrogenesis and ciliary reversal¹⁵ exhibit both types of receptor potentials on mechanostimulation¹⁶. Studies in wild-type *P. caudatum* (A. Ogura and H.M., unpublished) establish inward and outward mechanoreceptor currents and suggest that these currents are absent from the ciliary surfaces.

We conclude that activation of the ciliary motor response through mechanical stimuli involves the following steps: (1) increase in conductance of the somatic sensory channel; (2) passive spread of the receptor potential into the cilium; (3) conductance modification of the voltage-sensitive Ca^{2+} channel; (4) change in intraciliary Ca^{2+} concentration; (5) Ca^{2+} -dependent activation of the ciliary axoneme.

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JACQUES DE PEYER
HANS MACHEMER

Abteilung für Biologie,
Ruhr-Universität Bochum,
D-4630 Bochum, FRG

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Site of 1,25(OH)₂ vitamin D₃ synthesis in the kidney

FRASER AND KODICEK¹ have reported, and others have confirmed^{2–4}, that the kidney is the exclusive site of synthesis of 1,25-dihydroxycholecalciferol (1,25(OH)₂D₃). The 1- α -hydroxylation of 25-hydroxycholecalciferol (25-OH-D₃) occurs in mitochondria^{1,4,5} and is influenced by cytosol^{6,7}, although conflicting data have been reported⁸. Preparations of whole tubules from the cortex possess 1- α -hydroxylase activity¹⁰ but the precise localisation of the enzyme along the nephron is not known. In the dog, no evidence of 1- α -hydroxylase was found in medulla, enzymatic activity being observed only in cortical preparations⁵. In mice given systemic injections of ³H-vitamin D₃ autoradiography shows the tracer to be present in the proximal tubules⁹. However, whether hydroxylation occurred at this site was not established. Using single nephron preparations, we have now demonstrated that the major and probably exclusive site of synthesis of 1,25(OH)₂D₃ is the proximal tubule.

One-day-old white Leghorn cockerels were raised in the dark on a rachitogenic, vitamin D-deficient diet (ICN) containing 1.74% Ca and 1.24% inorganic phosphate (P_i). Controls of the same strain were maintained on a normal diet, in daylight. Rachitic chickens were distinguished from controls by their stunted growth, their weakness and the paucity of their feathers. Tubules were prepared by a modified technique of Imbert¹¹. After an intravenous injection of heparin, 42 64-day-old animals were bled, and the renal arteries were perfused via the aorta with a cold solution of collagenase (1 mg ml⁻¹) in modified Hank's solution containing 1.0 mM Ca. When completely blanched, the left kidney was removed. Thin sagittal slices were cut, from which minute fragments of tissue (0.5 × 0.5 × 3 mm), involving both superficial and deep parts of the kidney, were isolated and digested in collagenase solution (Type 1 from *Clostridium histolyticum*, Sigma) for 1 h at room temperature. Tubules were dissected at 0 °C, in Hank's modified solution.

The kidney of the chicken consists of three lobes, each divided into numerous lobuli. Each lobulus consists of a cortical part (under the kidney surface) containing nephrons which lack loops of Henle, and a medullary part containing nephrons which have short or long loops of Henle. A superficial nephron consists of a glomerulus, proximal tubule and distal tubule connected to the cortical collecting tubule^{12–15}. The early part of the proximal tubule (PCT), is convoluted, thick and relatively friable after digestion with collagenase. The PCT continues with a series of parallel hairpin loops, initially thick (thick loop cortex), and later thin (thin loop cortex), which finally connect to the branched collecting tubule (collecting cortex) through a thin distal convoluted tubule (DCT). The division between the DCT and the collecting cortex was difficult to distinguish so these two segments were dissected and tested together. Medullary nephrons were less readily digested by collagenase, and could be dissected as successfully as those of the cortex. Only small bundles of terminal collecting tubules in medulla were isolated satisfactorily, because of the resemblance of these terminal collecting tubules with the papilla in mammals, these structures were classified as papillary collecting tubules (collecting papilla).

After dissection, fragments of individual tubules were grouped according to their morphological characteristics until a total length of 4–16 mm per group was accumulated. Each group of tubules was kept at 0 °C in a drop of modified Hank's solution containing 0.25 mM of Ca, in the depression of a bacteriology slide. Tubules were photographed to measure their length and incubated in 0.6 to 1.0 μ l of incubation medium (IM) for 60 min at 35 °C. The final composition of the incubation medium was: malate 10 mM, Tris-HCl 100 mM, MgCl₂ 4 mM, EDTA 0.25 mM, glucose-6-phosphate 5 mM, NADP 0.33 mM, glucose-6-phosphate dehydrogenase 600 units l⁻¹, bovine serum albumin 1%. 25-Hydroxy-(26,(27)-methyl³H)-cholecalciferol (³H-25-OH-D₃, Roche), 95–110 Ci mmol⁻¹ was added to the preparation (the organic solvent for the labelled compound was evaporated under nitrogen previously). The 25-OH-D₃ concentration in the incubation medium varied from 135 to 560 nmol l⁻¹. After incubation, the contents of the slide depression were transferred to chromatography paper impregnated with silica gel (Whatman SG 81) and dried under nitrogen. The slide depressions were rinsed 4 times with 5 μ l of chloroform-methanol (1:2) and the washings were also transferred to the chromatography paper and dried. The chromatograms were developed in the dark for 16–18 h using chloroform-ethylacetate-benzene (40:50:10) according to the method of Bikle and Rasmussen¹⁰. They were then dried in air, cut into 2-cm²

Table 1 1,25(OH)₂D₃ synthesis along the nephron

Segment	25 OH D ₃ in IM (nmol l ⁻¹)	25 OH D ₃ recovery (fmol)	1,25(OH) ₂ D ₃ produced (fmol)	1,25(OH) ₂ D ₃ (fmol mm ⁻¹)	1,25(OH) ₂ D ₃ (fmol μ g ⁻¹ protein)	% Conversion
PCT (11)	334.6	89.7	0.22	0.03	0.20	—
	± 48.7	± 7.2	± 0.12	± 0.03	± 0.20	—
Thick loops cortex (5)	347.8	104.8	0.08	-0.01	-0.09	—
	± 77.7	± 14.0	± 0.03	± 0.00	± 0.06	—
Thin loops cortex (7)	358.1	108.7	0.00	0.01	0.09	—
	± 63.7	± 10.4	± 0.03	± 0.00	± 0.14	—
Distal and collecting cortex (9)	323.7	86.8	-0.03	-0.01	-0.13	—
	± 53.7	± 11.5	± 0.02	± 0.00	± 0.13	—
Collecting papilla (1)	203	73.2	—	—	—	—
PCT (19)	299.2	72.8	18.1	1.76	12.3	21.0
	± 29.4	± 1.7	± 2.0	± 0.19	± 1.4	± 2.3
Thick loops cortex (8)	261.6	78.3	12.8	1.73	12.4	14.3
	± 33.5	± 3.3	± 3.1	± 0.33	± 2.4	± 2.9
Thin loops cortex (7)	346.1	121.9	0.69	0.05	0.89	0.79
	± 66.6	± 19.6	± 0.61	± 0.06	± 1.00	± 0.60
Distal and collecting cortex (19)	356.0	116.4	0.16	0.02	0.28	0.37
	± 24.7	± 16.4	± 0.10	± 0.01	± 0.20	± 0.37
Collecting papilla (4)	256.0	84.2	0.43	—	—	0.01
	± 28.1	± 23.8	± 0.25	—	—	± 0.00

Values are means ± s.e.m. IM, incubation medium. Values in parentheses are numbers of groups of tubules tested.

pieces, and dispersed directly into scintillation vials containing 15 ml of toluene based scintillation fluid. The amount of vitamin D metabolites was expressed in fmol based on the original specific activity of the ^3H -25-OH- D_3 . Approximately 50% of the total radioactivity was recovered.

The chromatographic properties of the vitamin D metabolites were established using labelled authentic compounds: mean mobility of 1,25(OH) $_2$ - D_3 /25-OH- D_3 ratio was 0.50 ± 0.008 (s.e.m.). In six experiments with rachitic chickens, 10 μg each of carrier 25-OH- D_3 and 1,25(OH) $_2$ - D_3 were cochromatographed with the proximal tubule preparation, after incubation with ^3H -25-OH- D_3 . Unlabelled metabolites were localised on the chromatogram either by spraying with a solution of 10% phosphomolybdate in ethanol or by exposure to iodine vapour; the radioactive metabolites were then localised as previously described. The chromatographic properties of the authentic carrier sterols and the labelled metabolites formed by the tubules were identical (Fig. 1).

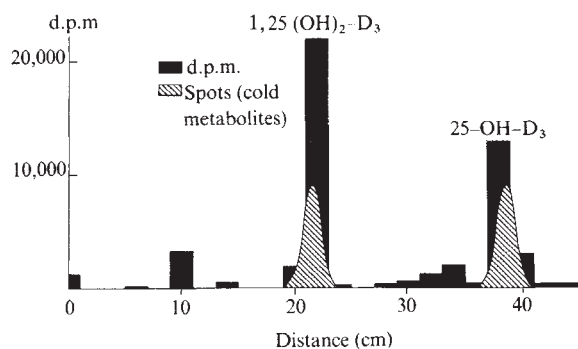


Fig. 1. Cochromatogram of a PCT preparation from a rachitic chicken after incubation with ^3H -25-OH- D_3 , and a mixture of the two unlabelled vitamin D_3 metabolites: 25-OH- D_3 and 1,25(OH) $_2$ - D_3 . The labelled metabolite produced by the tubular preparation exhibits identical migration characteristics to 1,25(OH) $_2$ - D_3 .

The mean protein content of the tubules was determined in three 10-week-old normal chickens, three 4-week-old normal chickens, and three 7-week-old rachitic chickens. Protein content was measured by a microtechnique described by Butcher and Lowry¹⁶. Fragments of tubules were processed and photographed as described above. As the protein contents of proximal convoluted tubules and thick loops of the cortex were similar, the results were pooled. Similarly, thin loops of the cortex, distal convoluted tubule, and cortical collecting tubules were combined and assayed together. Protein digestion in tubular preparations and in standards of bovine serum albumin was performed in 1 μl 6 M HCl, under oil, at 120 $^{\circ}\text{C}$ for 4 h. Fluorescence was developed by mixing the digested proteins with 100 μl of a mixture of 1 mM orthophthalaldehyde and 2 mM β -mercaptoethanol in 100 mM sodium tetraborate buffer at pH 10. The degree of fluorescence was measured after dilution in 500 μl of 0.5 M NaOH, using an Aminco-Bowman microfluorometer.

Plasma Ca and P_i were 3.33 ± 0.19 mEq l^{-1} and 1.55 ± 0.24 mmol l^{-1} respectively in rachitic chickens, compared to 4.07 ± 1.03 mEq l^{-1} and 2.61 ± 0.30 mmol l^{-1} in controls. The protein content of proximal (PCT thick loops cortex) and distal (thin loops cortex, collecting cortex) segments of the nephron were ($\mu\text{g mm}^{-1}$) 0.23 ± 0.4 and 0.11 ± 0.00 in 10-week-old chickens, 0.13 ± 0.00 and 0.06 ± 0.005 in 4-week-old chickens, 0.14 ± 0.01 and 0.06 ± 0.01 in rachitic chickens who were 5- to 9-weeks-old. Because of their size, 4-week-old chickens were used as controls, and the values of 1,25(OH) $_2$ - D_3 produced per mm tubule were converted, in the two series of animals, to fmol per μg of protein, utilising the corresponding tubular protein content.

The levels of 1- α -hydroxylase activity within the nephron segments are given in Table 1 and Fig. 2. The only significant production of 1,25(OH) $_2$ - D_3 occurred in the PCT and the thick loops of the cortex, in rachitic chickens: 18.1 ± 2.0 fmol and 12.8 ± 3.1 fmol respectively, that is, 1.76 ± 0.19 and 1.73 ± 0.33 fmol per mm of tubular length, or 12.3 ± 1.3 and 12.4 ± 2.4 fmol per μg protein. No 1,25(OH) $_2$ - D_3 was produced in the remaining nephron segments. The degree of 25-OH- D_3 transformation to 1,25(OH) $_2$ - D_3 varied between animals. The mean percentage transformation was $21.0 \pm 2.3\%$ and $14.3 \pm 2.9\%$ in PCT and the thick loops of cortex, with a maximum of conversion of 53%.

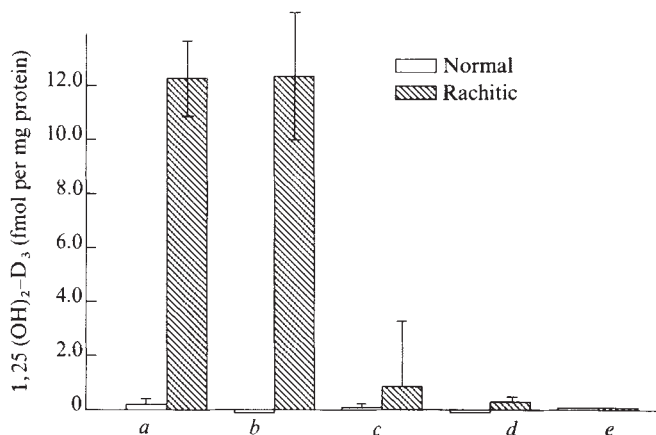
These studies demonstrate that, in vitamin D-deficient chickens, renal 1- α -hydroxylase activity is found exclusively in the proximal tubule. The absence of enzyme activity in the rest of the nephron may be assumed, unless other active segments are too short to produce detectable amounts of enzyme activity with this technique. If a distal site of enzyme activity exists, its activity is negligible in comparison with that found in the proximal tubule.

Our results agree with those reported by Midgett *et al.*⁵ who observed 1- α -hydroxylase activity in the cortex of the canine kidney but none in the medulla. In addition, the present data localises the site of the enzyme activity in the cortex, providing evidence that the enzyme is located exclusively in the proximal tubule.

Using homogenates of chicken kidney cortex, Midgett⁵ has reported a production of 10 pmol of 1,25(OH) $_2$ - D_3 per mg of protein after 2 h of incubation. Henry *et al.*¹⁷ using kidney homogenates has reported a production of approximately 1 pmol of 1,25(OH) $_2$ - D_3 per min per mg of protein. In the present study the proximal tubule exhibited a wide range of enzyme activity depending on age and on various other factors. The mean production of 1,25(OH) $_2$ - D_3 was 12.3 fmol per μg of protein during a 60-min incubation, a value which is of the same order of magnitude as those found in other laboratories.

The proximal tubule is responsible for most of the P_i reabsorption. In this segment, PTH¹⁸, and in some species, calcitonin¹⁹, stimulate cyclic AMP synthesis which interferes with P_i transport. PTH and cyclic AMP enhance the synthesis of 1,25(OH) $_2$ - D_3 (refs 20-23) and, conversely, vitamin D and its metabolites increase P_i reabsorption²⁴⁻²⁶, probably by interfering with adenyl cyclase activity²⁷. The localisation of 1- α -hydroxylase activity within the proximal tubule where the above factors converge their influence, would suggest that 1,25(OH) $_2$ - D_3 may participate in the regulation of tubular P_i transport.

Fig. 2 1,25(OH) $_2$ - D_3 synthesised by various segments of the nephron of normal and rachitic chickens. Columns indicate the mean values and bars the standard errors. A significant amount of the vitamin D_3 metabolite was found only in PCT and thick loops of the rachitic chickens. The results are expressed in fmol of 1,25(OH) $_2$ - D_3 per μg of tubular protein. a, PCT; b, thick loop cortex; c, thin loop cortex; d, DCT; e, collecting papilla.



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MICHELE G. BRUNETTE
MEANTHAN CHAN
CHRISTINE FERRIERE
KENNETH D. ROBERTS

Department of Pediatrics, Maisonneuve Hospital,
University of Montreal, School of Medicine,
Montreal, H1T 2M4 Canada

Received 14 July; accepted 12 September 1978.

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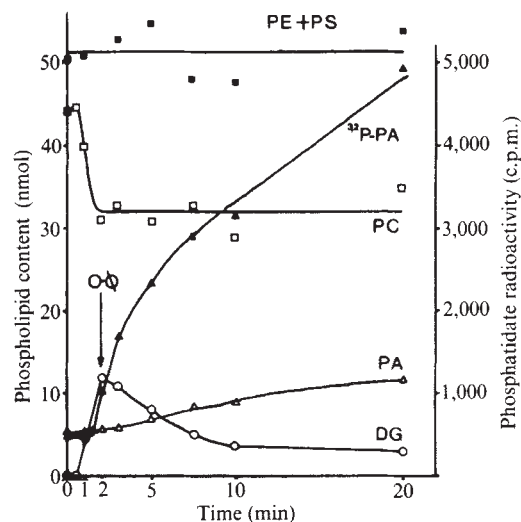


Fig. 1 Time course of production of 1,2-diacylglycerol and phosphatidate in human RBCs exposed to phospholipase C. Out-dated human RBCs were washed and prelabelled for 90 min at 37 °C with ³²P-Pi (10 µCi ml⁻¹) at a cell concentration of 2 × 10⁹ ml⁻¹ in a HEPES-buffered Ringer solution as described previously². Phospholipase C (C. Welchii freeze-dried culture filtrate type A, batch AD1051A, Wellcome, Beckenham, UK) was added at a final concentration of 10 µg ml⁻¹ and was inactivated after 2 min incubation by addition of *o*-phenanthroline to 1 mM final concentration; 0.5-ml samples of the incubation were taken at various times and immediately added to 1.9 ml of methanol:chloroform:concentrated HCl 200:100:1. Lipids were extracted as described previously and after concentration were separated according to the procedures of Skipski *et al.*¹² (phospholipids) and Freeman and West¹³ (neutral lipids). Phospholipids were quantified by the procedure of Bartlett¹⁴ following digestion with perchloric acid, and ³²P radioactivity in phosphatidate was measured by Cerenkov counting in a Searle liquid scintillation counter. The 1,2-diacylglycerol spots from the chromatogram were excised and heated at 100 °C for 1 h with 1 ml of methanol:concentrated HCl (10:1) under nitrogen in a screw-top tube. After cooling, the samples were dried at 40 °C under reduced pressure. The residue, which contained free glycerol, was silylated under N₂ at 100 °C for 10 min with a mixture of 20 µl pyridine and 20 µl *bis* (trimethylsilyl)-acetamide (Supelco, Bellefonte, Pennsylvania); 5 µl of the resulting mixture was applied to a 1.5-m GLC column (3% OV1 on 80–100 mesh Supelcoport) at an N₂ flow rate of 40 ml min⁻¹ and a temperature of 120 °C in a Pye 204 chromatograph. The silylated derivative of glycerol gave a peak at 3.8 min and was quantified by measurement of peak area compared with peaks given by various amounts of standard dipalmitin (Sigma) which had been treated in an identical fashion. Cell lysis was measured in samples of cells that were taken at various times and diluted in an isotonic saline solution containing 1% formaldehyde (to fix the cells) and 1 mM *o*-phenanthroline (which inhibits the phospholipase). The cells were spun down and the optical density at 410 nm of a suitable dilution of the supernate was measured. The experiment shown was one of five which gave closely similar results. ■, Phosphatidylethanolamine + phosphatidylserine; □, phosphatidylcholine; ○, 1,2-diacylglycerol; △, phosphatidate; ▲, phosphatidate radioactivity.

used this procedure, in which the endogenous diacylglycerol kinase of the erythrocyte is used to trap 1, 2-diacylglycerol appearing on the inner surface of the red cell membrane, to compare the time courses of phospholipase C-catalysed production of diacylglycerol and of the appearance of phosphatidate, and thus obtain an estimate of the rate of transbilayer migration of 1, 2-diacylglycerol.

Washed human RBCs were incubated with ³²P-phosphate to prelabel their ATP pool. They were exposed briefly to phospholipase C and the enzyme was then inactivated with *o*-phenanthroline (see Fig. 1). Samples were taken at various times and determinations made of 1,2-diacylglycerol, phosphatidate and phosphatidylcholine content. Radioactivity in phosphatidate was also measured.

Rapid transbilayer diffusion of 1,2-diacylglycerol and its relevance to control of membrane curvature

SHEETZ AND SINGER have proposed that the actions of certain amphipathic drugs in producing curvature of the membrane of human red blood cells (RBCs) could be explained by assuming that these drugs selectively entered one of the leaflets of the lipid bilayer, thus causing lateral expansion of that leaflet relative to the other¹. The membrane was therefore forced to curve in order to accommodate this extra material and the direction of this curvature was predictable, leading to production of either stomatocytes or echinocytes. Membrane curvature, leading to the formation of stomatocytes or echinocytes, can also be produced by subjecting human RBCs to phospholipase C attack^{2–5}. In this report we attempt to explain these phospholipase C-induced changes in terms of Sheetz and Singer's model, taking into account the observation that 1,2-diacylglycerol (the product of phospholipase C action), unlike phospholipids, can migrate rapidly across the membrane bilayer. Rapid transbilayer migration of 1, 2-diacylglycerol has previously been inferred from the observed increase in the rate of synthesis of phosphatidate in intact RBCs that have been exposed to phospholipase C: this phosphatidate is formed from diacylglycerol and cytosolic ATP by diacylglycerol kinase, presumably at the inner surface of the membrane². We have now

As shown in Fig. 1, there was a lag of about 30 s before the phospholipase began to attack the RBC lipids rapidly. Break-down of phosphatidylcholine and accumulation of 1,2-diacylglycerol then continued until the enzyme was inactivated with *o*-phenanthroline at 2 min. The maximal rate of phosphatidate synthesis was about $1.5\text{--}2.0\text{ nmol min}^{-1} 10^{-9}$ cells, and this corresponded to the maximum rate observed in other experiments when diacylglycerol kinase was assayed in lysed cells (unpublished) or in membranes⁶. In the experiment in Fig. 1, cell lysis was <5% in phospholipase-treated cells after 20 min, so that the great bulk of the 1,2-diacylglycerol must have initially been derived from outer-leaflet phosphatidylcholine and must then have traversed the membrane to gain access to diacylglycerol kinase and ATP. This assumption is supported by fatty acid determinations (unpublished) which showed that the compositions of 1,2-diacylglycerol and of phosphatidate derived from it, were not distinguishable from the composition of phosphatidylcholine. The maximum rate of synthesis of phosphatidate was observed about 1 min after achievement of the maximal rate of production of 1,2-diacylglycerol, suggesting that 1,2-diacylglycerol diffused into the inner leaflet of the cell membrane within about 1 min of its production in the outer leaflet. This rate is very much faster than the rates of transbilayer diffusion for erythrocyte phosphatidylcholine, the half-time of which for 'flip-flop' has been estimated as between 0.5 and 6.5 h (refs 7–9).

A schematic diagram to illustrate the possible morphological consequences of this fast diffusion of diacylglycerol is shown in Fig. 2. Formation of diacylglycerol from phospholipid in the

outer leaflet (Fig. 2b) is followed by rapid movement of diacylglycerol into the inner leaflet (Fig. 2c), thus causing a simultaneous contraction of the outer leaflet and expansion of the inner leaflet and a resulting tendency for the membrane to curve inwards. Phosphorylation of the internal 1,2-diacylglycerol (Fig. 2d) may further expand the inner leaflet as the utilisation of the diacylglycerol of the inner leaflet would favour the continued diffusion of diacylglycerol in from the outer leaflet. Also as phosphatidate would probably occupy a larger area than 1,2-diacylglycerol. However, the phosphorylation of diacylglycerol should not be the essential step in the induction of membrane curvature; it should be sufficient that phospholipid is broken down on one side of the membrane to give an accumulation of a rapidly migrating species such as diacylglycerol. This model therefore provides a rationale for the membrane invagination which follows attack on RBCs by externally added phospholipase C (ref. 2) and also for the evagination of membrane which follows activation by Ca^{2+} of the endogenous phospholipase C that attacks the polyphosphoinositide of the inner leaflet to give an accumulation of 1,2-diacylglycerol^{3–5}.

In both of these situations, the membrane curvature induced by asymmetric modification of the lipid bilayer brings membrane surfaces closer together and thus is an essential step which precedes the membrane fusion events which cause the release, either internally or externally, of membrane vesicles. As has been suggested before⁴, diacylglycerol-rich areas of membrane may promote the membrane fusion process, perhaps by formation of hydrophobic regions segregated from the bilayer¹⁰.

Although this model for the induction of membrane curvature by the unilateral conversion of phospholipids to diacylglycerol applies to the erythrocytes of man and some other species^{2–5}, similar principles might govern the control of membrane curvature and fusion in other physiological systems. The phospholipases C which have been identified in animal cells are activated by Ca^{2+} and act specifically on phosphoinositides^{3,11}, suggesting that inositol lipids, and the phospholipases C that attack them, may have an important role in the control of membrane curvature and fusion *in vivo*, especially in processes (for example, exocytotic secretion) that are brought about by an increase in the intracellular Ca^{2+} ion concentration.

We should also consider the possibility that conversion of phospholipids to other species which can rapidly cross a membrane bilayer (for example, fatty acids) may also affect membrane curvature in a similar fashion.

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DAVID ALLAN
PAUL THOMAS

Department of Experimental Pathology,
University College Hospital Medical School,
University Street,
London WC1 UK

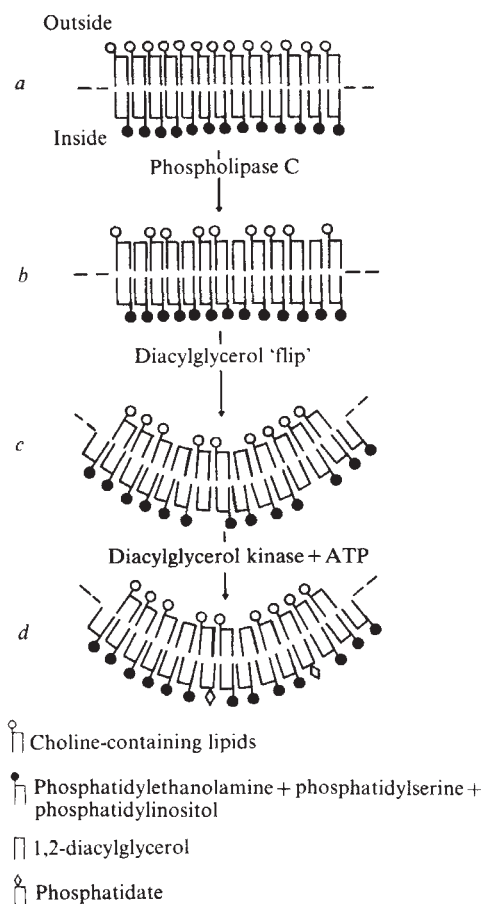
ROBERT H. MICHELL

Department of Biochemistry,
University of Birmingham,
P.O. Box 363,
Birmingham, UK

Received 20 July; accepted 3 October 1978.

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Fig. 2 Membrane bending induced by conversion of phospholipid to 1,2-diacylglycerol in one leaflet of a lipid bilayer, followed by diffusion of diacylglycerol into the other leaflet. *a*, Untreated bilayer; *b*, bilayer treated with phospholipase C on the outside; *c*, equilibration of diacylglycerol across the bilayer with consequent induction of bilayer curvature; *d*, phosphorylation of diacylglycerol to form phosphatidate on the inner face of the bilayer.



Whitepox virus isolated from hamsters inoculated with monkeypox virus

SINCE the eradication of smallpox in Equatorial Africa several 'new' pox viruses have been isolated in our laboratory from materials collected by WHO field workers—monkeypox virus from an affected individual and the 'whitepox' viruses from apparently healthy monkeys and rodents. Monkeypox virus is not widespread but occasionally infects man and has caused death: 33 such cases have occurred in Africa, with 6 deaths. It is not known whether whitepox viruses are pathogenic for man but by laboratory markers they are indistinguishable from variola virus¹. We report here that a whitepox virus identical to the whitepox virus found in feral monkeys and rodents in Equatorial Africa has now been isolated from the kidney and lungs of golden hamsters with an asymptomatic form of infection caused by intracordial inoculation with monkeypox virus. This result supports the hypothesis that natural whitepox viruses are white clones always present in populations of monkeypox virus.

Isolates from monkeypox virus population clones identical with whitepox viruses were shown to be stable and did not change their properties with serial passages on the chorioallantoic membrane of developing chick embryos². As cloning is an artificial laboratory procedure we attempted to determine whether this change in monkeypox virus population occurs in a living host. We suspected the existence of such a mechanism on the basis of whitepox virus detection in African rodents (*Mastomys natalensis*) insusceptible to this virus.

Three-week-old golden hamsters (*Mesocricetus auratus*) inoculated intracordially with 0.5 ml of chorioallantoic monkeypox virus culture (Copenhagen strain) at a dose of 10^6 pock-forming units (PFU) were used as an experimental model. For virus isolation from visceral organs groups of inoculated animals were killed 3 days post infection and at 7-day intervals. At each stage the blood and organs of 10 to 40 animals were tested. Suspensions of 1 part tissue: 2 parts McIlvain buffer solution were prepared from the liver, kidney, lung, spleen, lymph nodes, brain and testis. Viruses were isolated on the

chorioallantoic membrane (CAM) of 12-day-old chick embryos; incubation was for 72 h at 35°C. Pox antibodies in the serum were determined in haemagglutination inhibition (HAI) test (with 2 haemagglutination units of vaccinia virus), and in the neutralisation tests on chick embryos by pock reduction on the CAM using standard methods.

Hamsters inoculated with the virus intracordially as well as by intranasal and percutaneous routes showed no clinical symptoms of the infection. Pox antibodies were detected in nearly 50% of the infected hamsters. In animals in which antibodies were present they developed during the second week, reached the maximum by 4–5 weeks and disappeared 8–9 weeks post inoculation. Pox antibody titres were not high (the highest antihemagglutinin titre did not exceed 80).

Within the first 3 days after inoculation virus was found in the blood and organs of almost all the animals examined. A week later virus was isolated from only 30% of the animals. The number of virus carriers continued to decrease: after 2 weeks 25% carried the virus, after 4 weeks 13%, and after 6 weeks, 9%. At 7 and 9 weeks post infection no virus was isolated from the hamsters. It should be emphasised that two and more weeks post inoculation the virus could be detected only in lungs and/or kidneys of the hamsters and was absent from blood.

Investigation of isolates obtained from the hamsters' organs 3 days, 1 week and later post inoculation showed that in a majority of cases they were identical with monkeypox virus used for inoculation. However, 4 of 35 isolates differed markedly from the parent virus: three had been isolated from kidneys 2, 3½, and 4 weeks post inoculation and one from lungs of the hamsters 4 weeks post inoculation. A more detailed study showed these viruses to be fully identical with natural whitepox viruses isolated from African animals as well as with variola virus. A comparison of two of these isolates with the parental strain of monkeypox virus and whitepox virus Chimp-9 is shown in Table 1.

Further passaging of 'white' isolates on the CAM of chick embryos did not change their properties.

Our results show that 'white' clones can be produced from a monkeypox virus population by a 'natural' mechanism as well as by cloning. In asymptomatic infections of hamsters induced by

Table 1 Comparison of 'white' isolates derived from kidneys of hamsters infected intracordially with monkeypox virus

Markers		Isolates		Reference virus strains	
		MpW _{Ham-1}	MpW _{Ham-2}	Whitepox (Chimp-9)	Monkeypox (Copenhagen)
	Pock morphology†	White, dense	White, dense	White, dense	2 types: flat with haemorrhages and single white
Chick embryos*	Lethality LD ₅₀ (PFU per 0.1 ml)	2.3	2.5	2.2	<1
	Accumulation in liver† (PFU per g tissue)	8.5	8.3	8.7	9.1
	Pock formation at 39°C	None Positive	None Positive	None Positive	Yes Negative
PEK marker					
Neuropathogenicity for white mice§					
LD ₅₀ (PFU per 0.03 ml)		6.3	4.6	4.4	2.7
Rabbit skin test at inoculation (dose, 10^6 PFU per 0.1 ml)	Intradermally	Scarce soft infiltrate	Scarce infiltrate	Scarce infiltrate	Dense infiltrate with necrosis
	On scarified skin	No reaction	No reaction	No reaction	Papulopustular eruption

Pock morphology was determined on CAM.

* Age of chick embryos at inoculation—12 days.

† Incubation post inoculation—72 h at 35°C.

‡ PEK marker—presence of CPE and haemadsorption of chick erythrocytes of the continuous pig embryo cell line infected with virus dose of 10^4 PFU.

§ Neuropathogenicity was determined on 10-day-old white mice at intracerebral inoculation.

monkeypox virus the population of monkeypox virus that persists in the organism undergoes certain changes. It seems likely that 'white' clones initially present at a low level (<5%) are preferentially selected for. This selection process occurs in an unpredictable manner and the factors controlling it are not known. It seems probable that the whitepox viruses detected in feral animals (including those not susceptible to them) are produced through a similar mechanism.

Finally, as whitepox viruses are indistinguishable from variola virus in all laboratory marker tests our results may be considered as a first experimental indication of a possible origin of variola virus from monkeypox virus.

S. S. MARENNIKOVA
E. M. SHELUKHINA

WHO Collaborating Centre on
Smallpox and Other Poxvirus Infections,
Moscow Research Institute for Virology,
USSR Ministry of Health, Moscow, USSR

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Transcription of cloned *Xenopus* ribosomal genes visualised after injection into oocyte nuclei

DNA CLONING and the direct visualisation of chromatin transcription by electron microscopy provide useful details of the structure and function of genes, but the value of these techniques would be even greater if they could be applied to the same genes or segments of DNA. Our previous work¹ on amplified ribosomal DNA (rDNA) of the water beetle *Dytiscus* showed that rDNA injected into oocytes is correctly transcribed. Gradients of growing rRNA fibrils could be unambiguously recognised as correct transcription when compared to the characteristic transcription pattern observed in the original cell type. We have now extended the usefulness of this technique to

recombinant DNA, choosing as an example of a cloned vertebrate gene a single rDNA repeat of *Xenopus laevis* contained in plasmid DNA. The results show that this gene may be transcribed with remarkable fidelity in injected oocytes, and that untranscribed genes are converted into a characteristic chromatin structure.

A pMB9-based recombinant plasmid (pXlr 101) was used; its composition²⁻⁴ is summarised in Fig 1a. To determine the size

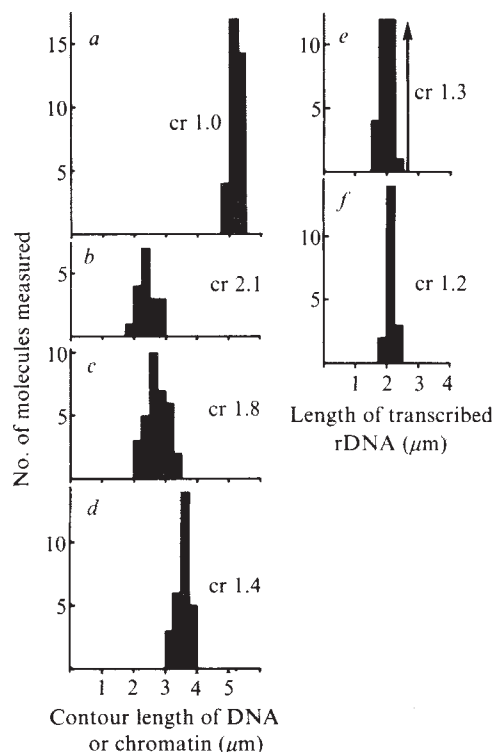
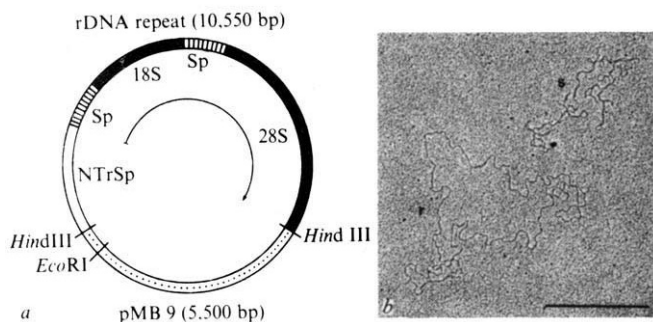


Fig. 2 Length distributions of DNA used for injection and of chromatin recovered from reisolated nuclei. *a*, DNA spread with cytochrome *c*. *b*, Beaded chromatin circles without apparent transcriptional complexes. *c*, Chromatin circles with sparse irregular transcriptional activity. *d*, Chromatin circles with fully transcribed regions, presumably the rDNA insert. The numbers designated *cr* in each histogram indicate the compaction ratio of DNA in the chromatin circles compared to the length of fully extended pure DNA (*a*). *e* and *f* show the lengths of the fully transcribed chromatin regions which carry transcripts. *e*, Chromatin circles from injected nuclei. *f*, Endogenous amplified rDNA. The arrow in (*e*) indicates the fully extended length of rDNA which can carry transcribed sequences. All measurements shown in *b-f* were made on the same single nuclear spread preparation.

Fig. 1 *a*, Diagram of pXlr 101. This is a single rDNA repeat of *Xenopus laevis* inserted into the plasmid pMB9 at the *Hind*III site. The rDNA repeat (10,550 base pairs) is orientated with the non-transcribed spacer (NTrSp, 2,350 base pairs) next to the *Eco*RI and *Hind*III sites of the plasmid. The rDNA coding region is limited at its 3' end by a *Hind*III cut 50 base pairs before the end of the normal 28S transcription region. Normal rDNA transcription initiates and terminates where indicated by the arrow inside the diagram. DNA has been characterised as described in ref. 4. *b*, Electron micrograph of circular recombinant DNA used for injection. DNA was spread out for electron microscopy as described before²⁷. The relaxed molecule (*r*) shown has a contour length of 5.06 μ m (see Fig. 2a). Most molecules occur in a supercoiled configuration(s). Scale bars, 0.5 μ m.



distribution of DNA used for injection, purified DNA was spread for electron microscopy (Fig. 1b). More than 90% of the molecules were in supercoiled circular configuration. Relaxed circles revealed a homogeneous distribution of contour lengths (mean value 5.19 μ m, Fig. 2a). To prepare nuclear spreads, oocytes from four *Xenopus* females were each injected with 50 nl DNA solution and a trace of ¹²⁵I-BSA, aiming for the nucleus⁵. Nuclei were isolated 8-18 h later, counted to identify successful nuclear injections, and spreads for electron microscopy prepared as before¹. Injected molecules were clearly identified in nuclear spread preparations by the small size of the injected circles¹. Almost all injected material was found as newly assembled chromatin^{6,7}, no large masses of 'naked' DNA being observed. Most of the beaded chromatin circles were seen as densely aggregated masses and could be individually identified only in the more dispersed peripheral parts of the preparations. Only clearly detached, open circular, molecules were measured and analysed further.

Our reasons for claiming that cloned rDNA can be treated normally in injected oocytes depend on its structure as tran-

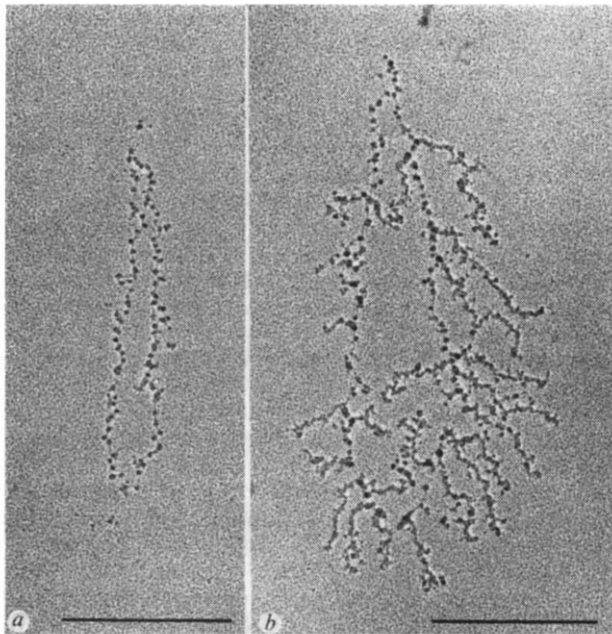


Fig. 3. Spread preparations of injected oocyte nuclei, showing circular chromatin. Oocyte nuclei were injected with purified DNA, incubated for 8 or 18 h (see text); individual nuclear contents were dispersed in low salt medium, and processed for electron microscopy with positive staining and rotary shadowing as before¹. *a*, A regularly beaded chromatin circle with no apparent transcriptional activity (contour length 2.43 μm). *b*, A typical example of the sparsely transcribed circle (contour length 3.10 μm). Note the irregular spacing of polymerases and the varying length of the lateral fibrils. These circles are taken from the same nuclear preparation as was used for histograms *b* and *c* of Fig. 2.

scribed or untranscribed chromatin. Injected DNA circles can be classified as non-transcribed or transcribed depending on whether lateral RNP fibrils are absent or present respectively.

The great majority of chromatin circles have a regularly beaded ultrastructure (Fig. 3*a*; 80–90 beads per circle; diameter of the particles after positive staining and rotary shadowing, 12–20 nm) and a mean contour length of 2.45 μm (Fig. 2*b*). This

suggests that the injected DNA is assembled into a regular nucleosome structure since it appears to have 180–200 base pairs DNA per nucleosome repeat (see ref. 8). There is a 2.1-fold compaction of the original DNA into chromatin as visualised after dispersion in low-salt medium. A similar compaction was obtained after injection of various other samples of circular DNA, including pMB9 DNA and a recombinant pMB9 plasmid containing four *Xenopus laevis* 5S DNA repeats (data not shown). These results are in agreement with calculations made for spread linear chromatin from various eukaryotic cell types^{9–11}.

The identification of transcriptionally active chromatin is based on the presence of nascent ribonucleoprotein (RNP) fibrils (see ref. 12 for limits of detection and refs 13, 14 for distinction between nucleosomes and RNA polymerases in nuclear spread preparations). Transcriptional complexes on injected circles were observed in 6 out of 21 preparations of spread nuclear contents. On each of these six grids more than 90% of the injected material was assembled into transcriptionally inactive chromatin. Transcriptionally active chromatin was classified as sparsely or densely transcribed according to the packing and arrangement of polymerases and the lengths of the attached RNP fibrils. We have seen about equal numbers of sparsely or densely transcribed chromatin circles. Sparsely transcribed chromatin (see Fig. 3*b* for a typical example) has a regularly beaded structure interspersed with a low number of irregularly spaced polymerases (3–10 polymerases per μm chromatin). The attached RNP fibrils show no graded length distribution and are up to 0.80 μm long. In some cases such circles contain short segments (0.2–1.2 μm) with closely packed polymerases (30–50 polymerases per μm) carrying transcripts of irregular length. The average contour length of these sparsely transcribed circles is 2.77 μm (Fig. 2*c*); this is greater than the mean length of the non-transcribed molecules.

Densely transcribed chromatin has at least 1.6 μm of closely packed polymerases bearing transcripts of gradually increasing length. We interpret the molecules shown in Fig. 4*a, b* as examples of correct transcription of cloned *Xenopus* rDNA. Molecules of this kind, of which we have measured 28 examples, show a detailed morphological similarity to the transcription units of endogenous amplified rDNA from the same nucleus (Fig. 4*c*). Circles of the kind shown in Fig. 4*a, b* are characterised by having three regions: (1) A densely transcribed region, which includes the 18S, 28S sequences. (2) A non-

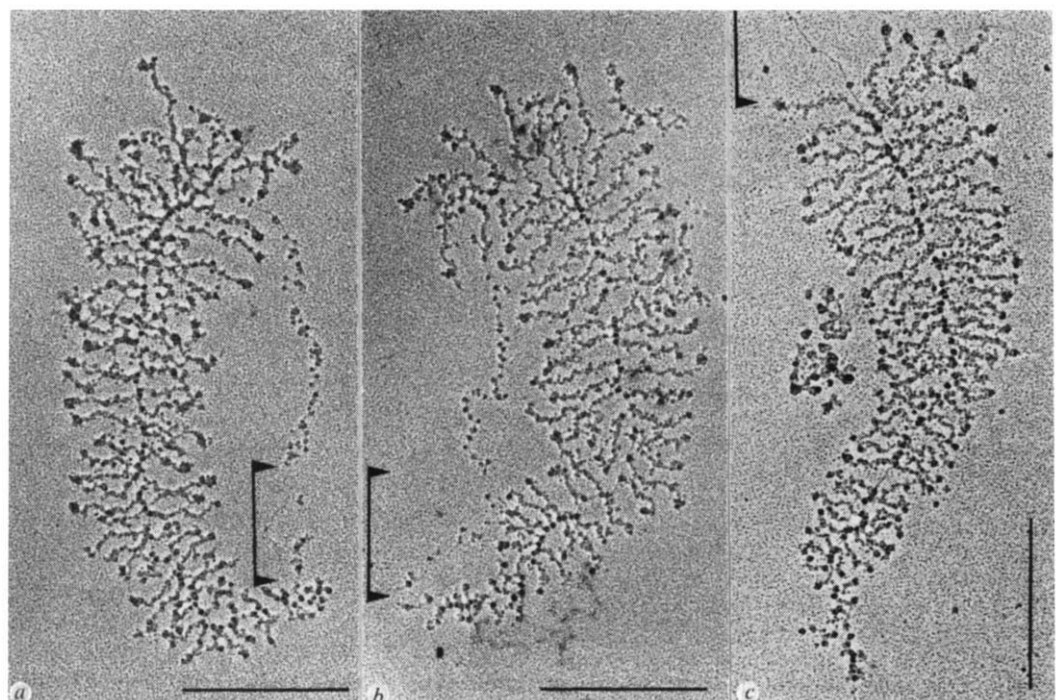


Fig. 4 Ribosomal DNA transcription patterns of injected recombinant plasmid circles (*a, b*) and an endogenous rDNA segment (*c*) seen in the same preparation. The morphology of the transcription units is identical by the criteria of density and size of rDNA-attached polymerases, the lengths and gradient arrangement of lateral fibrils, and their terminal coiling which results in dense, knob-like structures. Note the predominantly unbeaded structure of the 'spacer' segments in both figures (arrows).

transcribed stretch of DNA with a 'smooth' ultrastructure: this precedes the densely transcribed 18 + 28S region, and probably corresponds to the 'non-transcribed' spacer sequences. (3) A regularly beaded region without detectable transcriptional activity, presumably representing the pMB9 plasmid DNA. Within the transcribed regions, polymerases are densely packed (30–50 polymerases per μm), and lateral precursor rRNP fibrils are arranged in a typical length gradient, with terminal fibrils up to 0.40 μm in length frequently ending in knob-like structures. The length of chromatin which bears transcripts is 1.63–2.34 μm (mean 1.98 μm ; see Fig. 2e); this is conspicuously similar to the length of transcribed regions of endogenous amplified rDNA seen on the same preparations (range 1.90–2.39 μm , mean 2.14 μm ; see Fig. 2f) and reported in other studies^{2,15–19}. The 'spacer' segments of injected rDNA are seen as predominantly 'smooth' stretches (mean length 0.63 μm); they are located before the start region (5' end) of the transcribed regions, and are characterised by a greatly reduced number or complete absence of associated granular particles, in contrast to the beaded structure of the plasmid chromatin adjacent to the 3' end of the rDNA insertion.

Figure 2d shows that the circles containing densely transcribed rDNA (mean contour length 3.61 μm) are compacted 1.4 times compared to the length of the fully extended DNA before injection (Fig. 2a). This is considerably less compaction than is seen in non-transcribed or sparsely transcribed rings obtained from the same preparation (Fig. 2b, c). Circles with a densely transcribed rDNA insert allow a precise comparison of the structural configuration of DNA in transcriptionally active and inactive regions. The 8,200 base pairs of transcribed rDNA is 2.64 μm as pure DNA and 1.98 μm as transcribed chromatin; this represents compaction of 1.3, and shows that transcribed rDNA is almost fully extended (Fig. 2e, f). A similarly extended DNA configuration can be calculated for most of the smooth 'spacer' regions (see also refs 13, 14, 18, 20).

The precision with which injected rDNA may be transcribed is remarkable. The existence of a gradient of transcript lengths suggests that all initiations have taken place at the same point. The typical length of chromatin densely packed with polymerases indicates that transcription is terminating at the correct distance after initiation, as is also implied by the typical length of the longest transcripts. The nucleosome-free region of chromatin immediately in front of the initiation site is characteristic of rDNA transcription. We conclude that a single ribosomal gene repeat contains sufficient information in its DNA sequence to promote accurate transcription, and that this can take place on a relatively short length of DNA, as previously demonstrated biochemically for 5S genes²¹ and 4S genes^{22,23}. The transcription of the injected rDNA is resistant to low levels of α -amanitin, indicating that it is not transcribed by polymerase II; the accurate morphology of the densely transcribed rDNA suggests that it involves the use of polymerase I.

One of the most interesting general conclusions to emerge from the electron microscopy of nuclear spreads is that the transcription of ribosomal genes is not regulated simply by the supply of RNA polymerase, but seems to be governed by some component which makes a gene either wholly unavailable (closed) or maximally available (open) for polymerase attachment and transcription. Evidence for the existence of such a component has been implicated in previous nuclear spread studies. In amphibian oogenesis²⁴, as also in *Drosophila* spermatogenesis²⁵ and blastoderm embryos²⁶, most ribosomal genes are not transcribed, but a minority are maximally packed with actively transcribing polymerases. If ribosomal gene transcription were regulated by the availability of RNA polymerase molecules, most genes should have had a small number of widely spaced polymerases. The existence of a component which makes a gene open or closed for polymerase attachment is indicated very clearly in our own experiments reported here. By injecting about 10^5 genes into an oocyte, we are increasing by two to three orders of magnitude the endogenous ribosomal gene content of an oocyte nucleus (2×10^6 genes) and by 10^6 the normal content

of a somatic nucleus (10^3 genes). Therefore we certainly have an experimental condition in which genes greatly exceed the abundance of their polymerase. We would never expect to see the dense packing of polymerases on a gene (as shown in Fig. 4) in the absence of the postulated gene control component. The fact that we have used cloned genes ensures that all the injected DNA circles which we observe are equally capable of being transcribed. There is otherwise the possibility that some of the untranscribed endogenous genes may be genetically altered and incapable of transcription. Finally, the fact that we inject genes as purified DNA demonstrates particularly clearly that oocytes have available a limited supply of the ribosomal gene-controlling component. This experimental system can now be used to determine whether a similar component affects the transcription of non-ribosomal genes.

Thus, our results indicate that a single ribosomal gene cloned in bacteria can be correctly transcribed after the injection of recombinant plasmid DNA into oocyte nuclei. This encourages the use of injected oocytes to investigate the structure and function of unknown segments of chromosomal DNA which have been cloned in bacteria.

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M. F. TRENDELENBURG
J. B. GURDON

MRC Laboratory of Molecular Biology,
University Medical School,
Hills Road, Cambridge, UK

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Sequences from the genome of a non-transforming mutant of polyoma virus

POLYOMA, the small DNA animal tumour virus, like the SV40 virus, has attracted interest because its genome is small enough to be analysed at the molecular level^{1–6}, enabling study of the

molecular basis of oncogenic transformation. In polyoma virus there are several deletion mutants, designated *hr-t* (host-range transformation) mutants, which are unable to transform cells (or cause tumours) and which will grow in certain types of cells only, mainly those which have already been transformed. We report here that we have sequenced the region of the DNA which probably determines the non-transforming phenotype of one such *hr-t* mutant (NG-18)⁷ and compared it with the sequence of wild-type polyoma virus DNA. These studies show NG-18 to be a frame-shift mutant, and to have lost 187 of the nucleotides present in wild-type DNA, including several nucleotide triplets which code for cysteine.

NG-18, as well as other *hr-t* mutants, contains a deletion in the 'early region' of the polyoma virus genome^{8,9}. This region is thought to contain the coding information for at least three antigenically related proteins, which have been called 'small T-antigen', 'large T-antigen' and membrane-associated or 'middle T-antigen'. The early region probably contains the information required for transformation. The general properties of *hr-t* mutants are as follows: their genetic defect can be rescued⁸ by the polyoma virus wild-type restriction fragment *HpaII*-4, which maps between 78.4 and 91.6 units on the DNA physical map (see Fig. 1); the deletion in NG-18 has been more precisely defined to lie within a *HaeIII* subfragment of *HpaII*-4⁸. A protein the size of small T-antigen (molecular weight 21,000–22,000) is absent from cells infected with *hr-t* mutants^{9,10}, but a protein indistinguishable in size from the large T-antigen (MW ~ 100,000) is produced^{10,11}. *Hr-t* mutants and *ts A*-gene mutants (the *A* gene is thought to code for large T-antigen¹¹) complement each other for both transformation and virus production^{12,13}. Cells infected with *hr-t* mutants also seem to lack a protein (MW ~ 55,000) which occurs in wild-type lytically infected and transformed cells^{9,10}, is associated with

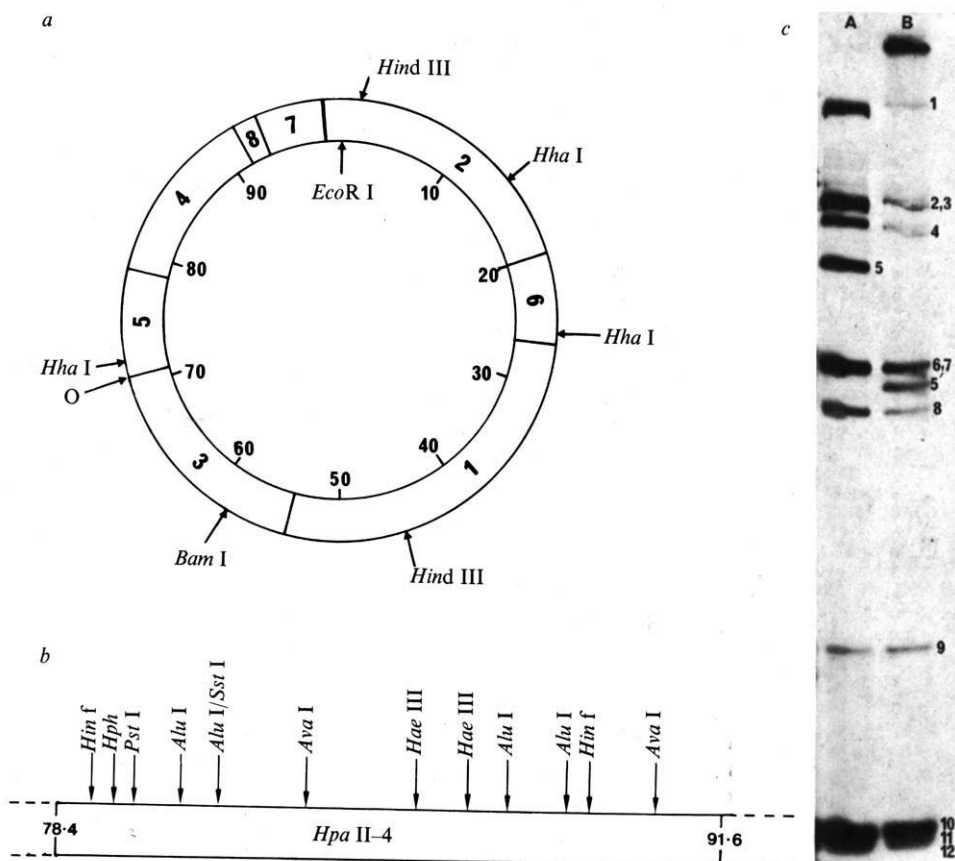
membranes¹⁶ and seems to contain some tryptic peptides in common with small T-antigen¹⁴.

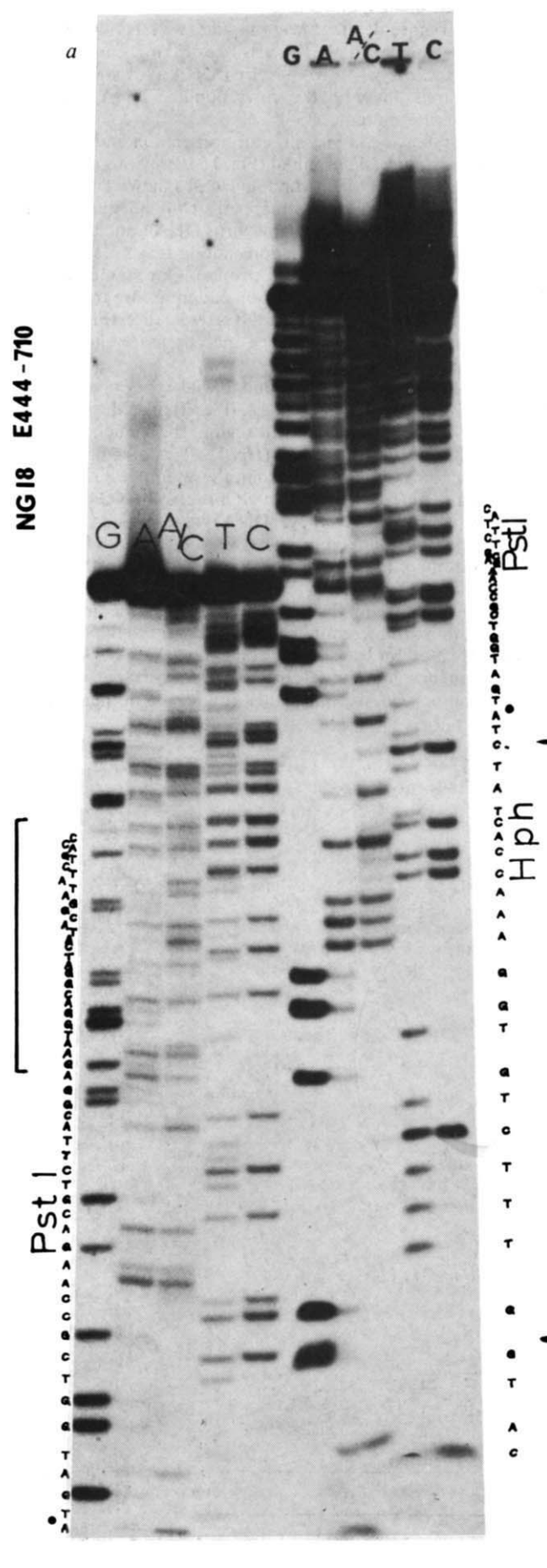
To look in more detail at *hr-t* mutants, we have determined the DNA sequences of the *HpaII*-4 restriction endonuclease fragment of wild-type polyoma virus DNA (A2 strain)¹⁵ and have compared this with the corresponding DNA sequence found in the *hr-t* mutant, NG-18.

Before sequencing, a fine-structure restriction endonuclease map of *HpaII*-4 was determined (Fig. 1b). Previous analysis of NG-18 DNA with restriction endonucleases showed it to have a deletion of about 3% which mapped at 80–85 units on the physical map of wild-type polyoma virus DNA⁴, and to lack the *SstI* and one of the *AvaI* restriction endonuclease sites found in *HpaII*-4 (M. Fried, unpublished results). Our studies showed that the fifth largest *HinfI* restriction endonuclease fragment in polyoma virus DNA contained the NG-18 deletion (Fig. 1c) and we used this fragment for sequence analysis across the deletion of the mutant DNA (Fig. 2).

A comparison of sequences found in wild-type polyoma virus DNA (A2 strain) and NG-18 DNA (Fig. 2) gave the following results. (1) Except for the deleted region, the sequences found between 78.4 and 91.6 map units (*HpaII*-4) in polyoma virus A2 DNA were indistinguishable from those found in the corresponding region of the DNA in NG-18. (2) In NG-18 DNA, 187 base pairs (relative to A2 DNA) were missing, and the deletion could be mapped to lie between 80.5 and 84 units on the physical map. (3) There are many sequences in the region between 79 and 86 map units (Fig. 3) which contain translational termination codons, but these are present in two reading frames only. The third frame contains no termination codons and is presumably used for translation. Following the deletion in NG-18, the sequence does not re-enter the reading frame which has no termination codons, but rather re-enters another reading

Fig. 1 a, The *HpaII* restriction endonuclease map (100 units) of polyoma virus DNA (A2 strain) showing the region containing the origin of viral replication (O) (taken from ref. 15). b, An expanded restriction endonuclease map of the *HpaII*-4 fragment. *HpaII*-4 contains the functional deletion found in the *hr-t* mutant, NG-18. The restriction endonuclease sites shown have been independently mapped (ref. 23, Fried, unpublished, and B. E. G., unpublished) and have also been confirmed by sequence analysis (ref. 3 also, see Fig. 3). c, An autoradiogram showing A2 (A) and NG-18 (B) DNAs which have been cleaved with the restriction endonuclease *HinfI* and separated by electrophoresis on a 5% acrylamide-bisacrylamide gel (20 × 40 cm)¹⁵. *HinfI* cleaves polyoma DNA into 12 fragments, the fifth largest of which is found in *HpaII*-4 (B. E. G., unpublished). This fragment, which is about 10.5% the size of A2 DNA, occurs as a fragment about 7% in size in NG-18. The NG-18 *HinfI*-7% fragment (band 5' in channel B) has been used to determine the sequences 'missing' in the mutant DNA (see Fig. 2). Cleavage of the ³²P-terminally labelled A2 *HinfI*-10.5% fragment with *HaeIII* gave new fragments about 7.0 and 2.5% in size; cleavage of NG-18 ³²P-terminally labelled *HinfI*-7% gave fragments about 3.5 and 2.5% in size. Sequences from the larger fragment in each case are shown in Fig. 2.



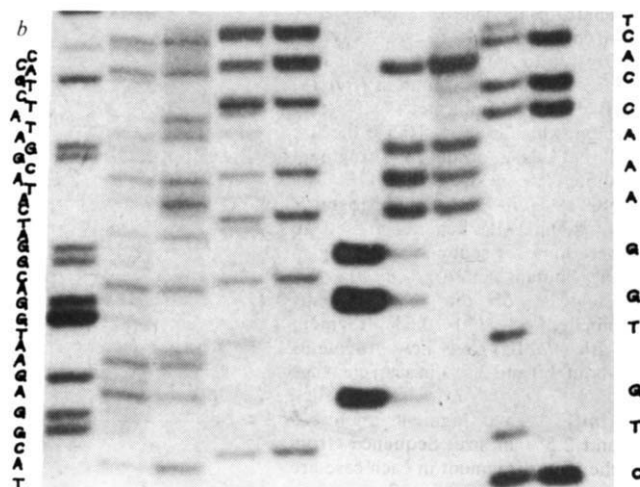


frame, and a termination signal is met within two codons of the deletion. (4) In the 'open' reading frame between 79 and 86 map units, the codon TGT occurs six times and TGC four times in wild-type polyoma DNA. These are cysteine codons. In NG-18 DNA, all but two of these codons are absent.

The data which concern the deletion of NG-18 are summarised in Fig. 3. What conclusions do these data allow? First, as the genetic defect in NG-18 was found to be rescued by the restriction fragment *Hpa*II-4 (ref. 8), and as, except for the deletion found in NG-18, our sequence analysis has shown the mutant and wild-type DNAs to be indistinguishable over this region, it can reasonably be assumed that the deletion is responsible for the mutant phenotype. Second, as NG-18 is essentially a frame-shift mutant, and as the sequences which immediately follow the deletion include a termination codon, any protein(s) induced by the mutant would be smaller than might be expected from a consideration of the size of the deletion alone. Third, the translation product expected from the wild-type DNA would contain a large number of cysteines, whereas a protein from the corresponding region of NG-18 would lack most of these cysteines. If this region of the genome of wild-type virus is coding for a functional protein, the removal of a large number of cysteines would almost certainly alter the conformation of the protein and would be expected to alter its function.

As the large T-antigen proteins made by NG-18 and polyoma wild-type DNA are indistinguishable in size^{10,11}, it is highly probable that the region of the DNA covered by the deletion is

Fig. 2 Polyoma virus DNA and DNA from the *hr-t* mutant NG-18 were cleaved with the restriction endonuclease *Hinf*I. Fragments were labelled at their 3' ends using [α -³²P]dATP and the other three cold deoxynucleotide triphosphates (TTP, dCTP and dGTP) in the presence of T4 polymerase²¹. The A2 *Hinf*-10.5% fragment and NG-18-7% fragment (Fig. 1c) were isolated, further cleaved with *Hae*III²³, and the sequences of the appropriate fragments (now labelled at one end only) were determined using the techniques described by Maxam and Gilbert²². *a-c* show autoradiograms of 20% acrylamide-bisacrylamide gels (20 × 40 cm) made essentially as described by Sanger and Coulson²⁴; samples were loaded on to the gels at two different time points. The sequence of more than 100 nucleotides can be read from these gels. *a*, The early strand (E) sequence (which contains information complementary to that found in the early mRNAs²⁵) from near the *Hinf*I site (79 map units) in NG-18 DNA to a position which corresponds to about 84.3 units on the physical map of polyoma virus DNA (A2 strain) (Fig. 1). (The sequence is underlined by a solid line in Fig. 3.) *b*, An enlargement of the part of *a* which indicates where the deletion occurs in NG-18 (between CAT and ACG in left half of the figure). *c*, The early strand sequences (E) from near the *Hinf*I site in wild-type polyoma virus DNA (A2 strain) to a position five nucleotides beyond the *Sst*I site (GAGCTC) at about 8.6 map units. This sequence is underlined by a dashed line in Fig. 3.

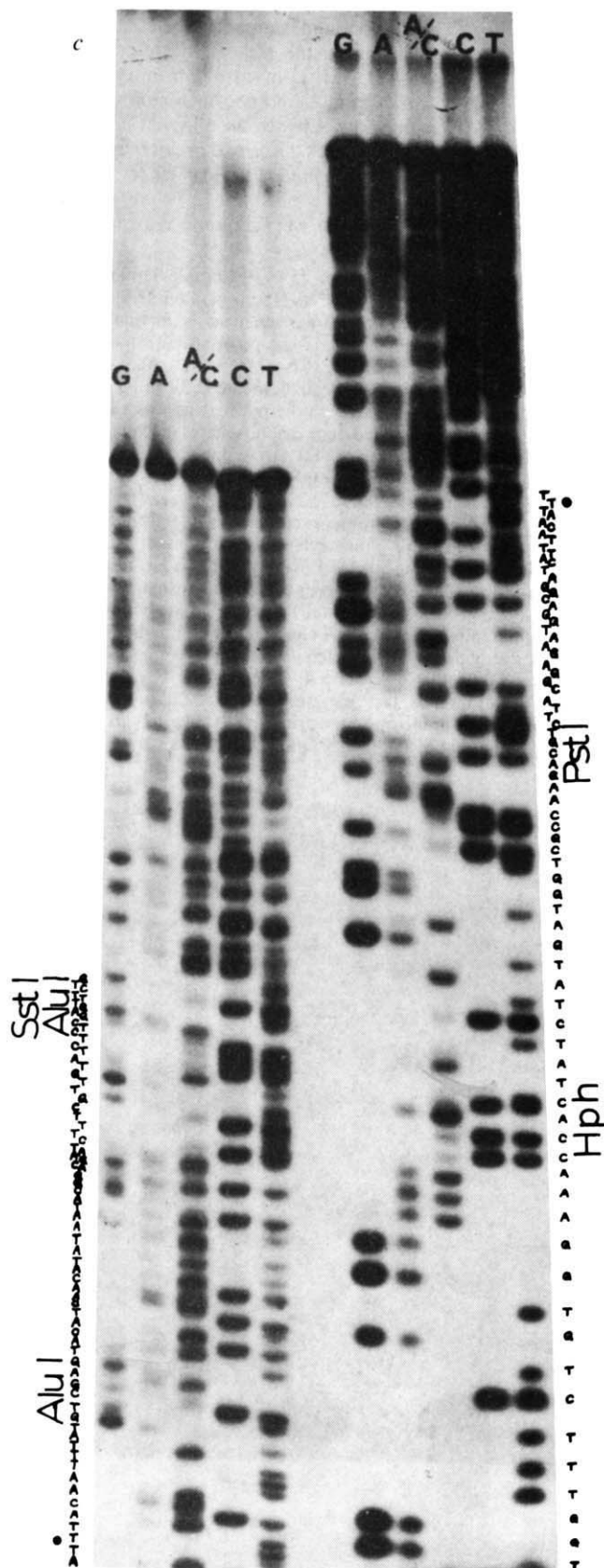


not a coding region for large T-antigen. This implies that in polyoma virus, large T-antigen is either coded for by sequences which occur after 84 map units on the physical map of the DNA (that is, clockwise from 84 map units, see Fig. 1) or large T-antigen is made from a 'spliced' mRNA, such as have been reported for SV40 and adenoviruses^{19,20}. The latter argument is supported by several facts: assuming large T-antigen to be entirely virally coded⁴, there is not enough coding information

left in the 'early region' beyond 84 map units to make a protein the size of large T-antigen. Moreover, among the early mRNAs of polyoma virus, there is an mRNA species that has a splice between 80 and 86 map units; this is postulated to be the mRNA for large T-antigen (R. Kamen, personal communication).

The polypeptide expected from translation of the sequences of polyoma virus DNA found between 79 and 86 map units is shown in Fig. 3b. These sequences show some homology (underlined) to sequences predicted to exist in the small T-antigen of SV40 (ref. 18). Although the homology is not extensive, the fact that it exists, that polyoma virus and SV40 seem to share a common overall organisation⁴, and that the NG-18 mutant (as well as other *hr-t* mutants) fails to induce small T-antigen, all suggest that sequences which lie between 80.5 and 84 units on the physical map of polyoma virus DNA code for a part of small T-antigen. It seems reasonable to assume, therefore, that small T-antigen may have a rôle in transformation.

Fig. 3 a, Some of the sequences found in the DNAs of wild-type polyoma virus (A2 strain and the *hr-t* mutant NG-18. DNA sequences designated 'late strand' correspond to sequences found in the early mRNAs²⁵. Sequence data for A2 DNA from about 79–86 map units are taken from gels shown in Fig. 2 or from data presented elsewhere³. (Numbers shown here and on gels in Fig. 2 represent the number of nucleotides from the *Hpa*II 3–5 junction in polyoma DNA which has been designated the 'zero-point' of the sequence.) NG-18 DNA sequence data are taken in part from the gel shown in Fig. 2. As the parent strain of NG-18 is not known, sequences from other parts of *Hpa*II-4 of mutant DNA were determined, data not given. No differences were seen between mutant and wild-type DNAs. Sequences found in A2 DNA but not in NG-18 DNA are shown within the brackets. Termination codons (TGA, TAG, TAA) are underlined. It can be seen that within this region of the wild-type DNA, only one frame can be used in translation if TGA, TAG and TAA are read as termination codons. The first termination codon found within the 'open' reading frame lies at about 86 map units and is indicated by a box. The protein(s) made from this region of the DNA can be seen to be remarkably cysteine rich (CYS). A protein induced by the NG-18 mutant should lack 8 of the 10 cysteine residues that can be predicted to be found in a protein induced by A2 DNA. b, The amino acid composition of a polypeptide predicted by the sequences shown in a. Amino acids shown inside the brackets would be absent in a protein translated from NG-18 DNA. Homologous regions between the predicted polypeptide of polyoma virus and small T-antigen of SV40 are underlined¹⁸; dashed lines show where very similar (ARG/LYS or LEU/ILE) sequences are observed.



The data obtained from sequencing NG-18 DNA do not allow this role to be assigned exclusively to either the cysteine-rich part of the protein or its normal C terminus, as both should be lacking in a protein induced by NG-18.

For SV40, several viable mutants have been isolated²⁶ in which deletions occur in a part of the SV40 genome which is comparable to the region in polyoma virus genome where the *hr-t* mutants have been mapped. These mutants fail to induce a protein the size of small T-antigen of SV40 (ref. 27) but do induce 'new' smaller proteins. Although the deletions in the SV40 mutants seem to have an effect on transformation, unlike the deletions in polyoma virus *hr-t* mutants, they do not entirely inhibit it^{28,29}. Further studies on other polyoma *hr-t* mutants and the SV40 mutants may therefore provide some insight into transformation.

One further point remains to be considered. No protein comparable to the membrane-associated (MW ~55,000) protein reported for polyoma virus¹⁶ has been found for SV40. As NG-18 also fails to induce this protein, it may also be coded for by sequences which lie between 80.5 and 84 map units on the physical map of polyoma virus DNA, and may also play a part in transformation. One of the next problems for study will be to determine whether the phenotypic differences between the polyoma virus and SV40 mutants are associated with small T-antigen or the membrane-associated protein, or possibly with both.

EIICHI SOEDA
BEVERLY E. GRIFFIN

Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London WC2, UK

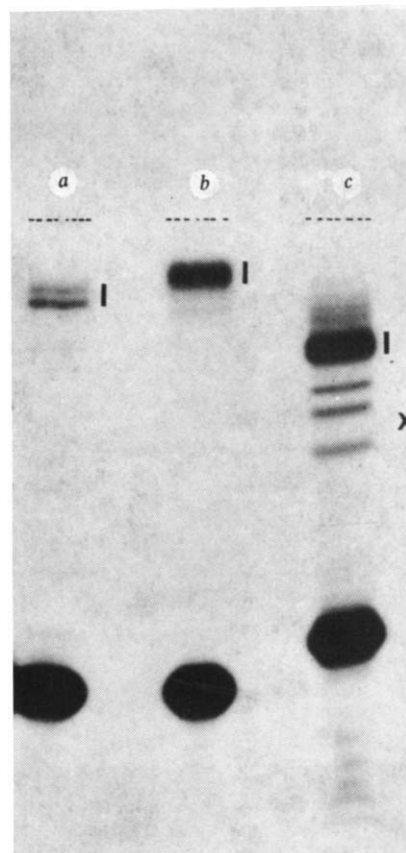
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We report here that, as a first step in identifying these sites we have sequenced between 33 and 156 nucleotides next to the 3'-terminal poly(A)¹²⁻¹⁴ of representative viruses from three of the four picornavirus genera¹⁵, the enteroviruses, cardioviruses and foot-and-mouth disease viruses (FMDV). The viruses chosen for these analyses allow us to make comparisons of the 3'-terminal sequences both within and between the three genera. Within each genus the 3'-terminal sequences are at least 60% homologous, whereas viruses from different genera show little similarity. The 3'-terminal sequences also show some unexpected features, including the lack of the putative signal sequence 5'-AAUAAA-3' (ref. 1) in viruses from two genera.

The virus RNA was partially¹⁶ copied into complementary DNA (cDNA) from the poly(A) with an oligo(dT)-containing primer and DNA polymerase I as described previously¹⁷, except that the cDNA was further purified (Fig. 1) so as to select only the larger DNA copies. These copies, not less than 43 nucleotides in length (primer included), were sequenced using the rapid

Fig. 1 Gel electrophoresis of [^{5'-³²P}] complementary DNA synthesised with the RNA of *a*, SVDV; *b*, poliovirus; *c*, FMDV, type SAT 2. The cDNA was synthesised in an incubation¹⁷ (1 h at 37 °C) containing: virus RNA (2-20 µg) purified as described elsewhere³², Tris-HCl pH 7.5 (67 mM), MnCl₂ (0.5 mM), β-mercaptoethanol (0.13 mM), NaCl (0.16 mM), [^{5'-³²P}] p(dT)₁₀dG or [^{5'-³²P}] p(dT)₈dC (~100 pmol) purified as described previously¹⁷, all four unlabelled deoxytriphosphates (each 0.67 mM), actinomycin D (50 µg ml⁻¹) and DNA polymerase I, Klenow fragment (4-8 units). Subsequently, the cDNA was isolated by phenol/chloroform extraction and ethanol precipitation. The transcription products were then separated by electrophoresis for 2-3 h at 900 V in a 15% polyacrylamide (0.6% bisacrylamide) gel (20×40 cm) containing 50 mM Tris-borate, 1 mM EDTA and 7 M urea, as shown. The bands migrating more slowly than the xylene-cyanol FF marker (X), indicated by a bar, were visualised by autoradiography and the cDNA eluted into water by homogenisation (4 extractions), then precipitated in a volume of 3.0 ml containing 0.05 M NaAc pH 5.4, 60 µg carrier RNA and 75% ethanol. The intense, fast migrating bands at the bottom of the diagram contain unincorporated primer, [^{5'-³²P}] p(dT)₈dC in lanes *a* and *b*, and [^{5'-³²P}] p(dT)₁₀dG in lane *c*.



3'-Terminal nucleotide sequences in the genome RNA of picornaviruses

IN common with eukaryotic cellular mRNAs¹⁻³, the terminal sequences in virus RNAs contain untranslated regions of variable length⁴⁻¹¹. These regions are likely to contain binding sites for macromolecules involved in functions such as replication⁴ and translation⁴⁻⁶ of the RNA, and assembly of virus particles.

chemical sequencing technique for $[5'-^{32}\text{P}]\text{DNA}$ (ref. 18). Figure 2 shows polyacrylamide gels displaying sequences complementary to the 3'-terminal regions of several picornavirus RNAs. The clarity of the band patterns indicates that transcription occurred exclusively from the poly(A), and allows 3'-terminal sequences to be read without difficulty. The 3'-terminal sequence of encephalomyocarditis virus (EMCV) RNA has previously¹⁷ been determined up to position 129 by the chemical method, and also in part by independent DNA and RNA sequencing techniques which indicated that the chemical method is reliable. In the present study, the chemically determined 3'-terminal sequences of poliovirus RNA (type 1, Mahoney strain) and FMDV RNA (type A, strain 61) have also been checked by other methods (not shown). In the case of poliovirus RNA, the sequence between positions 10 and 39 has been verified by the dideoxynucleotide method¹⁹, with reverse transcriptase as the copying enzyme and a dideoxynucleotide to deoxynucleotide ratio of 2.5:1. The 3'-terminal sequence of 20 nucleotides in the FMDV RNA was verified using the 'plus-minus' method^{20,21}.

The few nucleotide sequence uncertainties were, as expected, near the 3'-end of the cDNA (Fig. 3), where gel resolution was not perfect. One anomaly was observed in poliovirus cDNA (not shown) in that at nucleotide 92, a dG and a dC apparently occur in the same position. This may be due either to anomalous gel mobility caused by strong secondary structure¹⁹ or to heterogeneity in nucleotide sequence in one position²².

The 3'-terminal sequences for nine picornaviruses, determined in at least two independent experiments, are shown in Fig. 3. In all polyadenylated RNAs previously examined^{1,3,23,24} (except possibly Rous sarcoma virus RNA²⁵), the sequence 5'-AAUAAA-3' is located in a similar position in the untranslated region near the poly(A). This sequence has been implicated as a signal in polyadenylation¹⁷ as it is absent from plant virus RNAs, which are not polyadenylated⁸. The same A-rich hexanucleotide is also present in EMCV (ref. 10) and maus-Elberfeld virus (MEV) RNAs near the poly(A), but is absent from the 3'-terminal region in the two enterovirus and five FMDV RNAs examined here (Fig. 3). (Although GAUAAA is present in swine vesicular disease virus around position 40, its presence must be considered fortuitous, as it is located much further from where AAUAAA is normally found¹⁻³, and is absent from the corresponding region of poliovirus RNA.) The absence of an AAUAAA sequence near the 3'-end in the RNAs of the enteroviruses and FMDVs, all of which are polyadenylated, therefore brings into doubt the suggestion¹⁷ that this A-rich sequence is a universal signal for polyadenylation.

In the cardioviruses, a cluster of seven nonsense triplets occurs near the poly(A), with a frequency much greater than would be expected in a random sequence. At least two nonsense triplets block all three protein synthesis reading frames¹⁰, suggesting this as a possible device for preventing protein synthesis continuing into the poly(A) as a result of a frameshift mutation, or suppression of the natural termination codon of the coding region. Nonsense triplets are almost as numerous in the enteroviruses, though more widely spaced. As with the cardioviruses, they occur in the three possible reading frames, but with only two of the frames blocked more than once. Inspection of the positions of the nonsense triplets in all the picornavirus RNAs allows the minimum number of untranslated nucleotides next to the poly(A) to be deduced. The highest number so far (43) is in serotype SAT 2 of FMDV.

Unexpectedly, we found that two classes of cDNA are synthesised with the RNA of the C997 strain of FMDV (Fig. 2). The larger cDNA (C997(L)) is ~60–70 nucleotides long and is very similar in sequence to the p(dT)₁₀dG-primed cDNAs from four other FMDV RNAs (Fig. 3). The smaller cDNA (C997(S)) is ~43–53 nucleotides long and is identical in sequence to C997(L) in the 20 nucleotides next to the oligo(dT) primer; then, in complete contrast to C997(L), there is a run of at least 13 dA residues, giving a total of 17 consecutive dA residues (Fig. 2). Therefore, there seems to be an oligo(U) tract in some C997

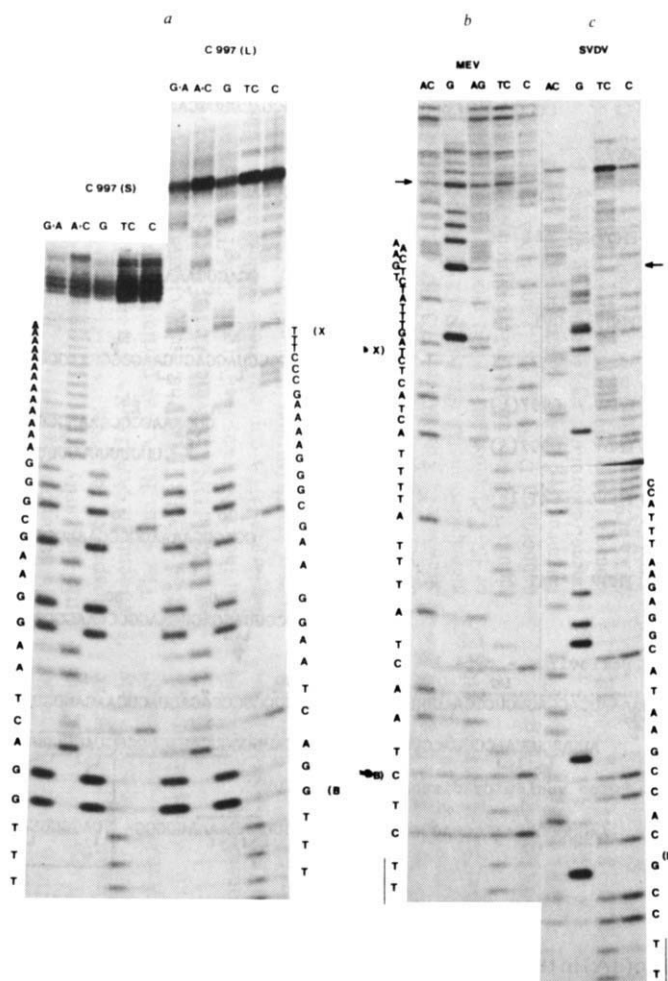


Fig. 2 Sequencing gels of *a*, $[5'-^{32}\text{P}] \text{p(dT)}_{10}\text{dG}$ -primed cDNA synthesised with FMDV RNA, strain C997; *b*, $[5'-^{32}\text{P}] \text{p(dT)}_{10}\text{dC}$ -primed cDNA from maus-Elberfeld virus RNA; *c*, $[5'-^{32}\text{P}] \text{p(T)}_{10}\text{dC}$ -primed cDNA from SVDV RNA. The $[5'-^{32}\text{P}]$ cDNA eluted from the polyacrylamide gel (Fig. 1) was divided into four or five equal volumes and each aliquot subjected to one of the six base-specific partial chemical degradations¹⁸ (dG > dA, dA > dG, dA > dC, dG, dT > dC or dC). The gel electrophoretic separation of the partial products was carried out as described¹⁸, with the following slight modifications: the gel (30 × 80 cm) was the same composition as that shown in Fig. 1; the samples were loaded in 5 mM Trisborate, 7 M urea, 0.1 mM EDTA, 0.05% bromophenol blue and xylene-cyanol FF, and 20% sucrose; the top buffer compartment contained 7 M urea; the gel was pre-run for 16 h at 1 kV, and electrophoresis was for 24–48 h at 1.3–1.8 kV. Autoradiography was for 7–21 d at -20°C . The prefix d for deoxy has been omitted.

virus RNA molecules near the poly(A). An alternative explanation is that the oligo(dA) is an artefact resulting from slippage of the DNA polymerase on reaching a short run of U residues in the template, but this is considered unlikely, for once DNA synthesis has been initiated, slippage is not known to occur with DNA polymerase. It is also unlikely that slippage would occur on only some template molecules. Preliminary RNase T₁ two-dimensional fingerprinting experiments with C997 virus RNA have shown the presence of a spot, the position of which would agree with both the size and composition of the oligo(U) tract detected in the sequencing studies (D.N.B. and K. J. H. Robson, unpublished data).

Oligo- or poly(U) has not been found previously in the messenger strand of any virus, although it seems to be present at the 5' end of the poliovirus minus strand²⁶, where it functions as the complement of the poly(A) on the opposite (plus) strand. It is not known whether oligo(U) has a special function in C997, nor whether it is present in other C serotypes. We have not detected

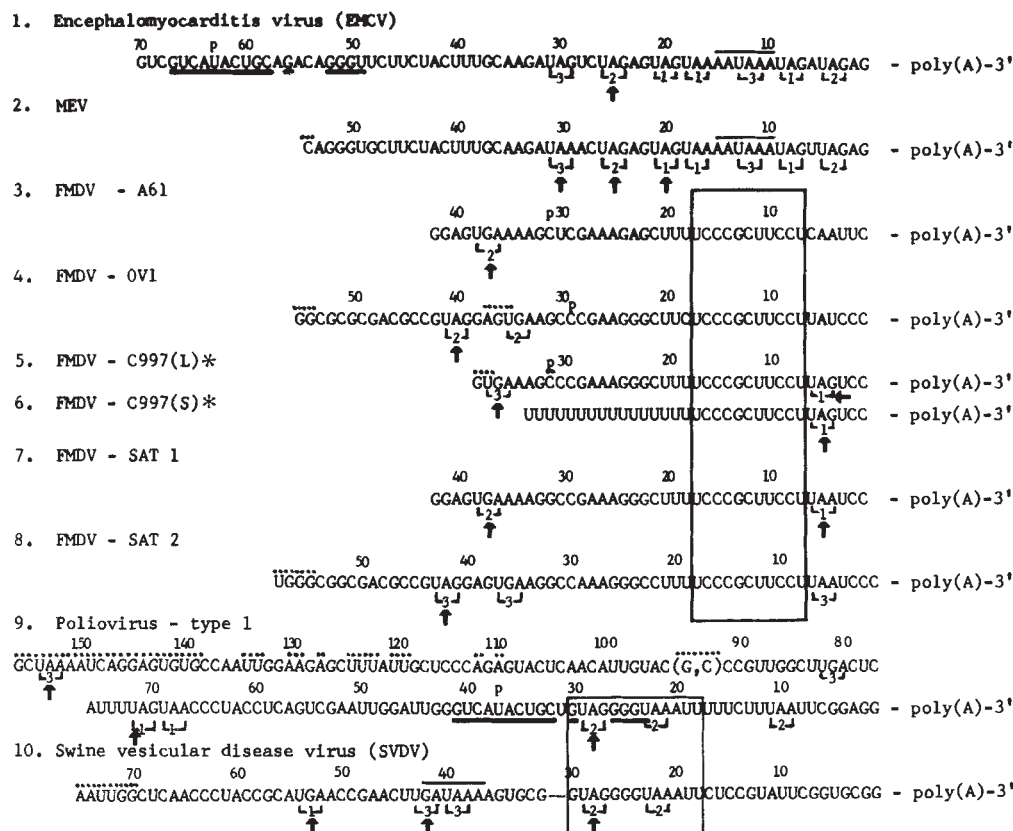


Fig. 3 3'-Terminal sequences of nine picornavirus RNAs. The sequences were deduced from the band patterns on the gels (Fig. 2). Slight uncertainties are indicated by a dotted line. In the enteroviruses and FMDVs, the highly conserved areas are boxed in. A thick line draws attention to the homology between poliovirus and EMCV RNA. A thin line shows the sequences AAUAAA and GAUAAA. The letter p indicates palindromic sequences. Nonsense triplets are bracketed, and the protein synthesis reading frames in which they occur are indicated. Arrows point to potential termination codons of the coding region. Note that in EMCV the single potential termination codon was assigned from the positions of the other nonsense triplets in the sequence of 129 nucleotides next to the poly(A) (ref. 17). *L and S refer to sequence derived from large or small cDNA.

oligo(dA) in the cDNA transcribed from the RNAs of four other FMDV serotypes, although the possibility of its presence in poorly transcribed cDNA cannot be ruled out.

There is considerable conservation of 3'-terminal sequences within the cardiovirus and FMDV groups (Fig. 3). The two cardioviruses are >90% homologous in the 54 nucleotides next to the poly(A). The five FMDV serotypes are ~75% homologous in the 20 nucleotides next to the poly(A), and this includes a highly conserved sequence of 11 consecutive nucleotides, which is indicated in Fig. 3. Excluding the RNA corresponding to C997(S) cDNA, all five serotypes of FMDV also show ~75% homology in the 38 nucleotides next to the poly(A). In this region, a purine-rich sequence (20 nucleotides) precedes a pyrimidine-rich sequence (20 nucleotides), suggesting that stable secondary structure could exist. For example, in the A61 strain, nucleotides 16-24 paired with nucleotides 29-37 would give a thermodynamically stable structure ($\Delta G \approx -8.4$ kcal mol⁻¹) (ref. 27). No such structure is likely in the C997 RNA species containing oligo(U), in which the 3'-terminal 33 nucleotides are almost entirely pyrimidines. There is, however, an opportunity for an alternative secondary structure in this RNA in which the oligo(U) pairs with the poly(A). The serologically unrelated enteroviruses, poliovirus and swine vesicular disease virus, are >60% homologous in the 71 nucleotides adjacent to the poly(A). Figure 3 draws attention to a highly conserved region in these virus RNAs. In SVDV, nucleotides 25-35 could pair with nucleotides 54-64, giving a highly stable double-stranded region²⁷ ($\Delta G \approx 12.0$ kcal mol⁻¹) in which seven out of 11 nucleotide pairs are GC. Only a marginally stable secondary structure could exist, however, involving the corresponding sections of poliovirus RNA.

Homologies between viruses from different genera are much less frequent. Figure 3 indicates one significant homology between poliovirus and EMCV, in which 15 out of 19 nucleotides are identical, between positions 49 and 67 in EMCV and positions 23 and 41 in poliovirus. It is interesting that much of

the homologous region has the same nucleotide sequence when read in either direction¹⁷ (a palindrome). 11- or 13-nucleotide palindromes are also present in the RNAs of the FMDV strains A61, OV1 and C997, centred at or near position 30. Palindromes have been found previously in the 3'-untranslated regions of host mRNAs from several different sources^{24,28-30}, and it has been suggested that they may provide binding sites for oligomeric proteins in the corresponding DNA³¹. However, this cannot be the case for the picornaviruses, which do not go through a DNA intermediate in their replication.

In conclusion, the presence of many nonsense triplets near the 3' terminus of some picornavirus RNAs, the absence of the putative signal AAUAAA in viruses from two genera and evidence of oligo(U) in a fraction of RNA from one virus, are all unexpected findings in the picornavirus genome. As expected, at least part of the 3'-terminal region in all picornavirus RNAs so far studied is not expressed into protein. It is possible that this untranslated portion has a special function, for example, to bind the virus polymerase complex during initiation of replication. Support for the idea that the 3' end may be specifically recognised comes from our finding that extensive sequence homologies exist in the RNAs of viruses within each picornavirus genus, whereas viruses from different genera show little similarity. These findings also strongly support the accepted scheme¹⁵ for the division of picornaviruses into genera.

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A. G. PORTER

P. FELLNER

Searle Research Laboratories,
Lane End Road, High Wycombe,
Bucks, UK

D. N. BLACK
D. J. ROWLANDS
T. J. R. HARRIS
F. BROWN

The Animal Virus Research Institute,
Pirbright, Woking, Surrey, UK

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DNA sequence of the bacteriophage λ *cI* gene

THE *cI* gene of bacteriophage λ codes for the λ repressor. This protein is both a positive and a negative regulator of gene transcription (see ref. 1 for review). These functions are mediated by the interaction of repressor with two operators in the phage DNA. Both the nucleotide sequence of the λ operators^{2–5} and the amino acid sequence of the λ repressor have been determined⁶. The ability of the λ repressor to bind operator DNA is greatly reduced by a proteolytic cleavage which occurs, for example, during *recA*-dependent prophage induction in *Escherichia coli*⁷. A single mutation (λ *cI* *ind*[–]) prevents this cleavage and inactivation of repressor^{7,8}. I present here the nucleotide sequence of the λ *cI* gene and identify the base change responsible for the *ind*[–] phenotype of λ repressor.

Restriction endonuclease fragments containing *cI* DNA were isolated either from phage λ DNA or from the plasmid pKB252 (ref. 9) by gel electrophoresis¹⁰, and were end-labelled and sequenced by the chemical method of Maxam and Gilbert¹¹. A partial restriction map of *cI* and surrounding DNA, and the positions and strands of sequenced restriction fragments are shown schematically in Fig. 1. Restriction fragments have been aligned by direct DNA sequence overlaps. The nucleotide sequence of *cI* and the amino acid sequence of the λ repressor⁶ are shown in Fig. 2.

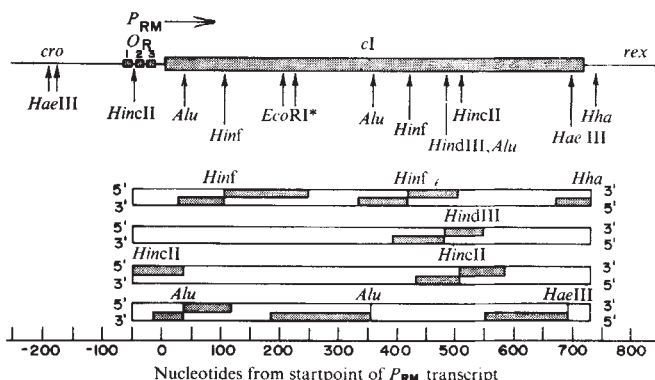
The sequence 5' CCAGG 3' occurs twice in the sense strand of *cI* DNA (bases 99–103 and 529–533). The second cytosine in this sequence is normally present as 5-methylcytosine in wild-type strains of *E. coli*^{12,13}. As 5-methylcytosine fails to give a band in the chemical DNA sequencing method^{11,14}, its location is usually deduced by the presence of a characteristic gap in the pattern of bands, combined with sequence analysis of the complementary DNA strand. The presence of 5-methylcytosine at base 100 of the *cI* sequence was confirmed by these methods. The assignment of base 530 as 5-methylcytosine relies on a comparison of the protein and DNA sequences. The DNA sequence read from the sequencing gel was 5' GAG C₅₃₀ A GGT 3', whereas the corresponding λ repressor sequence is Glu¹⁷⁵–Pro¹⁷⁶–Gly¹⁷⁷. The genetic code requires that proline be coded by a CCN codon, and therefore I assume that base 530 is present as 5-methylcytosine. Bases 102 and 532 of the *cI* antisense sequence are also present as 5-methylcytosines. In the *lacI* gene, 5-methylcytosines have been identified as hot spots for spontaneous and 2-aminopurine induced mutagenesis¹⁵.

The DNA sequence of *cI* (Fig. 2) confirms the amino acid sequence determined for λ repressor by independent protein-sequencing methods⁶. The nucleotide sequence also agrees with the partial sequences determined previously for the 5' and 3' termini of *cI*^{4,5,16} and for the 5' sequence of *cI* mRNA transcribed from the *P*_{RM} promoter^{1,16}.

The *ind*[–] *cI* mutation was sequenced from the *Hinf*/*EcoRI** 199-base-pair fragment (Fig. 1) isolated from phage λ DNA carrying the mutant allele. *ind*[–] *cI* DNA has an A at base 352 replacing the wild-type G. This base change generates an additional *HindIII* recognition site at bases 352–357 of *cI*. The presence of this additional restriction site in λ *cI* *ind*[–] DNA has been noted elsewhere¹⁷. The *ind*[–] base change occurs approximately halfway through *cI*, a position which correlates extremely well with its location in the genetic map constructed by Lieb¹⁸. In *ind*[–] λ repressor, a lysine should replace the wild-type glutamic acid at amino acid 117. The mutant repressor is thus considerably more basic than the wild type in physiological conditions.

The codon usage for wild-type *cI* is given in Fig. 3. Overall, there is a slight preference for T and A in the third base of the codons. However, the third position distribution is biased by the 11 codons in which G is obligatory (ATG and TGG) and the 50 codons for Lys, Glu and Gln which require either an A or G in the third position. In those triplets where any of the four bases is acceptable (CTN, GTN, TCN, CCN, ACN, GCN, CGN and GGN) there is a marked preference for T (38.6%) and a discrimination against G (10.9%) in the third position.

Fig. 1 Partial restriction map of *cI* and surrounding DNA. Restriction sites used for DNA sequence analysis of *cI* are indicated. Not all sites for *EcoRI**, *Alu* and *Hha* are shown. The hatched areas in the lower scheme indicate the extent of reliable DNA sequence information from particular restriction cuts. Restriction fragments were 5' labelled with [γ -³²P]ATP and polynucleotide kinase¹¹, and were cut with a second restriction enzyme. All sequencing is thus 5' → 3'. The upper strand is the *cI* sense strand.



NH₂-SER-THR-LYS-LYS-LYS-PRO-LEU-THR-GLN-GLU-GLN-LEU-GLU-ASP-ALA-ARG-ARG-LEU-LYS-ALA-
 ATG AGC ACA AAA AAG AAA CCA TTA ACA CAA GAG CAG CTT GAG GAC GCA CGT CGC CTT AAA GCA
 TAC TCG TGT TTT TTC TTT GGT AAT TGT GTT CTC GTC GAA CTC CTG CGT GCA GCG GAA TTT CGT
 10 20 30 40 50 60
 ILE-TYR-GLU-LYS-LYS-LYS-ASN-GLU-LEU-GLY-LEU-SER-GLN-GLU-SER-VAL-ALA-ASP-LYS-MET-
 ATT TAT GAA AAA AAG AAA AAT GAA CTT GGC TTA TCC CAG GAA TCT GTC GCA GAC AAG ATG
 TAA ATA CTT TTT TTC TTT TTA CTT GAA CCG AAT AGG GTC CTT AGA CAG CGT CTG TTC TAC
 70 80 90 100 110 120
 GLY-MET-GLY-GLN-SER-GLY-VAL-GLY-ALA-LEU-PHE-ASN-GLY-ILE-ASN-ALA-LEU-ASN-ALA-TYR-
 GGG ATG GGG CAG TCA GGC GTT GGT GCT TTA TTT AAT GGC ATC AAT GCA TTA AAT GCT TAT
 CCC TAC CCC GTC AGT CCG CAA CCA CGA AAT AAA TTA CCG TAG TTA CGT AAT TTA CGA ATA
 130 140 150 160 170 180
 ASN-ALA-ALA-LEU-LEU-ALA-LYS-ILE-LEU-LYS-VAL-SER-VAL-GLU-GLU-PHE-SER-PRO-SER-ILE-
 AAC GCC GCA TTG CTT GCA AAA ATT CTC AAA GTT AGC GTT GAA GAA TTT AGC CCT TCA ATC
 TTG CGG CGT AAC GAA CGT TTT TAA GAG TTT CAA TCG CAA CTT CTT AAA TCG GGA AGT TAG
 190 200 210 220 230 240
 ALA-ARG-GLU-ILE-TYR-GLU-MET-TYR-GLU-ALA-VAL-SER-MET-GLN-PRO-SER-LEU-ARG-SER-GLU-
 GCC AGA GAA ATC TAC GAG ATG TAT GAA GCG GTT AGT ATG CAG CCG TCA CTT AGA AGT GAG
 CGG TCT CTT TAG ATG CCG TAC ATA CTT CGC CAA TCA TAC GTC GGC AGT GAA TCT TCA CTC
 250 260 270 280 290 300
 TYR-GLU-TYR-PRO-VAL-PHE-SER-HIS-VAL-GLN-ALA-GLY-MET-PHE-SER-PRO-GLU-LEU-ARG-THR-
 TAT GAG TAC CCT GTT TTT TCT CAT GTT CAG GCA GGG ATG TTC TCA CCT GAG CTT AGA ACC
 ATA CTC ATG GGA CAA AAA AGA GTA CAA GTC CGT CCC TAC AAG AGT GGA CTC GAA TCT TGG
 310 320 330 340 350 360
 PHE-THR-LYS-GLY-ASP-ALA-GLU-ARG-TRP-VAL-SER-THR-THR-LYS-LYS-ALA-SER-ASP-SER-ALA-
 TTT ACC AAA GGT GAT GCG GAG AGA TGG GTA AGC ACA ACC AAA AAA GCC AGT GAT TCT GCA
 AAA TGG TTT CCA CTA GCG CTC TCT ACC CAT TCG TGT TGG TTT TTT CGG TCA CTA AGA CGT
 370 380 390 400 410 420
 PHE-TRP-LEU-GLU-VAL-GLU-GLY-ASN-SER-MET-THR-ALA-PRO-THR-GLY-SER-LYS-PRO-SER-PHE-
 TTC TGG CTT GAG GTT GAA GGT AAT TCC ATG ACC GCA CCA ACA GGC TCC AAG CCA AGC TTT
 AAG ACC GAA CTC CAA CTT CCA TTA AGG TAC TGG CGT GGT TGT CCG AGG TTC GGT TCG AAA
 430 440 450 460 470 480
 PRO-ASP-GLY-MET-LEU-ILE-LEU-VAL-ASP-PRO-GLU-GLN-ALA-VAL-GLU-PRO-GLY-ASP-PHE-CYS-
 CCT GAC GGA ATG TTA ATT CTC GTT GAC CCT GAG CAG GCT GTT GAG CCA GGT GAT TTC TGC
 GGA CTG CCT TAC AAT TAA GAG CAA CTG GGA CTC GTC CGA CAA CTC GGT CCA CTA AAG ACG
 490 500 510 520 530 540
 ILE-ALA-ARG-LEU-GLY-GLY-ASP-GLU-PHE-THR-PHE-LYS-LYS-LEU-ILE-ARG-ASP-SER-GLY-GLN-
 ATA GCC AGA CTT GGG GGT GAT GAG TTT ACC TTC AAG AAA CTG ATC AGG GAT AGC GGT CAG
 TAT CGG TCT GAA CCC CCA CTA CTC AAA TGG AAG TTC TTT GAC TAG TCC CTA TCG CCA GTC
 550 560 570 580 590 600
 VAL-PHE-LEU-GLN-PRO-LEU-ASN-PRO-GLN-TYR-PRO-MET-ILE-PRO-CYS-ASN-GLU-SER-CYS-SER-
 GTG TTT TTA CAA CCA CTA AAC CCA CAG TAC CCA ATG ATC CCA TGC AAT GAG AGT TGT TCC
 CAC AAA AAT GTT GGT TTT GGT GTC ATG GGT TAC TAG ACG TTA CTC TCA ACA AGG
 610 620 630 640 650 660
 VAL-VAL-GLY-LYS-VAL-ILE-ALA-SER-GLN-TRP-PRO-GLU-GLU-THR-PHE-GLY-COOH
 GTT GTG GGG AAA GTT ATC GCT AGT CAG TGG CCT GAA GAG ACG TTT GGC TGA TCG
 CAA CAC CCC TTT CAA TAG CGA TCA GTC ACC GGA CTT CTC TGC AAA CCG ACT AGC
 670 680 690 700 710

Fig. 2 DNA sequence of *cl* and amino acid sequence of λ repressor. The subscripted numbers represent the number of bases from the start of the *cl* P_{RM} transcript. Cytosines at bases 100 and 530 of the sense sequence, and 102 and 532 of the antisense sequence are present as 5-methylcytosines (see text).

		SECOND BASE					
		T	C	A	G		
FIRST BASE	T	PHE 8 PHE 4 LEU 6 LEU 1	SER 3 SER 4 SER 0 SER 0	TYR 4 TYR 3 STOP 0 STOP 0	CYS 1 CYS 2 STOP 1 TRP 3	T C A G	
	C	LEU 8 LEU 2 LEU 1 LEU 1	PRO 6 PRO 0 PRO 8 PRO 1	HIS 1 HIS 0 GLN 2 GLN 10	ARG 1 ARG 1 ARG 0 ARG 0	T C A G	
	A	ILE 3 ILE 6 ILE 1 MET 8	THR 0 THR 5 THR 4 THR 1	ASN 6 ASN 2 LYS 12 LYS 5	SER 5 SER 6 ARG 5 ARG 1	T C A G	
	G	VAL 11 VAL 1 VAL 1 VAL 2	ALA 4 ALA 5 ALA 9 ALA 1	ASP 5 ASP 4 GLU 9 GLU 12	GLY 6 GLY 5 GLY 1 GLY 5	T C A G	

Fig. 3 Codon usage for wild-type *cl*. The overall third position base frequencies are T, 30.5%; C, 21.1%; A, 27.1%; G, 21.1%. In those codons where any of the four bases in the third position specify the same amino acid (see text) the frequencies are T, 38.6%; C, 22.7%; A, 27.7%; G, 10.9%.

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ROBERT T. SAUER

Biological Laboratories,
Harvard University,
Cambridge, Massachusetts 02138

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reviews

New ecology exhibition at BM

Peter D. Moore

Introducing Ecology. British Museum (Natural History) Exhibition. *Nature at Work*. By British Museum (Natural History). Pp. 96. (Cambridge University Press: Cambridge, 1978.) Hardback £6.50; paperback £2.50.

AFTER about fifty years of conceptual evolution, the idea of the ecosystem, involving close relationships between the living and non-living world, is finally having an impact at the level of elementary biology teaching. This is very welcome, for it may result in a more precise understanding of the fundamental basis of modern ecology and restore some meaning to the very term. The exhibition recently launched at the British Museum (Natural History), together with the associated book, will certainly serve to encourage this laudable trend.

The exhibition and the book concentrate on the energy relationships of ecosystems, which has the advantage of providing a unifying theme for the concepts portrayed but which inevitably results in the neglect of other interesting aspects of ecosystem study. Unity and flow, however, are vital if the interest of a visitor is to be maintained and this is certainly achieved. But energy is not easily conceived; an early display tries to overcome this by encouraging the observer to turn a wheel on the exhibit with sufficient gusto to generate the electricity for lighting the panels. Great fun for young children, but I wonder whether it really helps with their thermodynamics?

Energy movement through ecosystems as a theme also generates the problem of how to explain photosynthesis, the primary level of energy conversion. Here the exhibition and the book are at their weakest, and understandably so. One is left with two options: either you try to explain the complex mechanism of photophosphorylation or you leave photosynthesis as a black box with sunlight going in and energy-rich foodstuffs coming out. In fact the former course was chosen. Chloroplast structure is explained in some detail and the production of ATP and NADH is mentioned, but chlorophyll itself remains something of a black box. It is rather strange that this degree of biochemical

detail is used at this stage when no equivalent account is given of respiration, a process just as vital to an understanding of energy flow. Also, as the emphasis throughout is on the presentation of concepts in a simple, pictorial fashion, is there any need to introduce such technical terms as autotrophs, heterotrophs, or even carbohydrates? Many of the weaker brethren my stumble needlessly at this first hurdle.

One of the unfortunate consequences of the energy flow concept from the botanical point of view is that the plant is reduced to the level of an energy conversion and storage system on which the rest of the (far more interesting) organisms depend. When photosynthesis has been dealt with, plants can be set on one side. Admittedly, some attempt is made to show the influence of climate and soil conditions on plant growth, but this display is difficult to follow because of its complexity.

From this point onwards, the quality of both exhibition and book begin to rise. The ways in which consumer organisms make a living are described and attention is drawn to the energy which their way of life demands in such activities as seeking food. Two habitats are considered in detail: an oak woodland (in which some taxonomic confusion over the species of oak involved is evident) and a rocky seashore (in which *Fucus serratus* is found above *Pelvetia canaliculata*). In dioramas of these habitats, the student is presented with such problems as working out food webs. In the book the answers are supplied at the end, but in the exhibition electronic links can be made between different organisms which results in a red or green light appearing, depending on the likelihood of one consuming the other. Observations on secondary school children operating this display suggested that many were unaware of the implications of the red or green light but this, I suppose, is one of the problems which educationists have to face.

Decomposition is tackled in a novel and effective way using animated flicker cartoons which are operated by the observer. The consequent recycling of elements is not fully dealt with at present, but I gather that a display of

the carbon cycle will be added to the exhibition later.

Perhaps the most ambitious and, in my view, the most successful aspect of energy flow to be considered is the energy pyramid and ecological efficiency. Those students who have worked their way this far without losing the thread are presented with such fascinating questions as why carnivores may be more efficient than herbivores, and why the pyramid of biomass in aquatic systems is an inversion of that found on land.

Watching children in the exhibition one soon comes to the conclusion that the most popular exhibits are those with which they can interact, where questions are asked, choices made and buttons pressed. In this respect a new dimension is attained at this exhibition. Having completed the exhibition circuit, the student meets a computer console and screen with which he can tackle an ecological question. A problem is set up by the computer, such as what factors control winter moth caterpillars in oakwoods. Several alternative solutions are presented and the operator makes a choice which results in the presentation of data either supporting or negating the selection. Rejection of that hypothesis then returns the student to the initial choices. The game provides a unique opportunity for a student to work his way through an ecological problem in a systematic and scientific fashion.

Presentation of material in both exhibition and book is clear, graphic and caricatured, sometimes to the extent of being bizarre. This will offend the purist. But whatever the dusty academic may think of this venture, the fact remains that on the one hand a seven year old child may find the demonstrations rivetting in their overt appeal and on the other there are questions raised here which still occupy the minds of research workers. The potential spectrum of interest is vast and the message is clear and basic, that ecology has moved beyond natural history and has become a unified science through the development of such concepts as energy flow. □

Peter D. Moore is Senior Lecturer in Ecology at King's College, University of London, UK.

Embryological cookery book

Methods in Mammalian Reproduction. Edited by J. C. Daniel. Pp. 566. (Academic: New York, San Francisco and London, 1978.) \$39.50; £25.65.

TEXTS that tell you how to do things, whether they be cookery books or manuals of bee-keeping or motor-cycle maintenance, can be valuable both for the experienced and for the novice; they also have a particular fascination for the voyeur, who has no intention of actually keeping bees or maintaining motor-cycles, but likes to identify with the contortions of those who do. Even the best of such books can be dangerous, however, if they are accepted as an alternative to visiting the master, watching him perform, and then trying the procedure yourself under his critical eye.

With this caveat, *Methods in Mammalian Reproduction* is warmly to be welcomed. Its predecessor, *Methods in Mammalian Embryology*, which appeared in 1971 with the same editor but a different publisher, must have achieved an extremely high average Citation Index rating; my own copy is constantly borrowed. The present volume has a very similar mix of 'developmental' and 'reproductive' topics to the previous one, despite its title, but the themes are now a little more sophisticated. For example, induction of ovulation has been succeeded by *in vitro* oocyte maturation, fertilisation by parthenogenesis, aggregation chimeras by injection chimeras.

Most of the book is concerned with embryos and the remarkable things that can be done to them during the pre-implantation period (oocyte maturation, experimental parthenogenesis, manipulation of ploidy, injection chimeras, immunological interference with development) or subsequently (embryo culture during and after implantation, embryo grafts to the chick, limb regeneration). Others describe techniques that can be applied more generally during development (freezing, tissue separations, one- and two-dimensional gel electrophoresis, and fluorography). Then there are chapters on particular groups of animals (marsupials; primates; embryo culture in rabbits, sheep and cows; embryo transfer in farm animals). Finally, there are a few rather heterogeneous chapters on the maternal side, dealing with pheromones, experimental delay of implantation, female genital tract secretions, intra-uterine devices in monkeys and rodents, grafting endometrium to ectopic sites, laparoscopy (not in humans) and

amniocentesis (only in humans).

Some chapters are long and very detailed: gel electrophoresis; injection chimeras; amniocentesis, including cell culture and chromosome banding. Some read more as interesting accounts of rather esoteric research fields than as descriptions of widely used techniques: endometrial transplantation; immunological inhibition of development (mainly with antisera against zona pellucida); regeneration of mammalian limbs (they don't, even with electrical stimulation, but bits of brain implanted in the stump help, and bone, muscle and nerve individually do quite well).

Bibliographies are satisfactory only up to 1976, with some 1977 papers listed as being in press; the descriptions of some rapidly advancing areas, such as the use of ultrasound and amniocen-

tesis in prenatal diagnosis, tend in consequence to be a little dated. Editing could be improved: for example, techniques for the recovery of embryos from monkey reproductive tracts are distributed among three chapters, laparoscopy, IUDs, and primates; no cross-references are given. The index is inadequate.

These are minor criticisms. If you have found Joe Daniel's *MME* useful, you will want to possess *MMR* also. The initial purchase price is high, but you are likely to recoup it many times over, by increased efficiency, avoidance of costly errors, and some appreciation of what you may be letting yourself in for.

Anne McLaren

Anne McLaren is Director of the MRC Mammalian Development Unit, London, UK.

Dioxin workshop

Dioxin: Toxicological and Chemical Aspects. Edited by F. Cattabeni, A. Cavallaro and G. Galli. Pp.222. (SP Medical and Scientific Books/Spectrum: New York and London; 1978.) Distributed by Halsted/Wiley. £14.50; \$26.50.

ON 10 July, 1976 the north Italian town of Séveso was contaminated with the discharge from a chemical reactor used for the manufacture of trichlorophenol. The discharge consisted primarily of sodium trichlorophenolate, but also contained an extremely toxic by-product of the reaction 2,3,7,8-tetrachlorodibenzodioxin (TCDD).

Faced with a serious pollution problem the Province of Milan and the Lombardy Regional Government sponsored a workshop in October 1976 to which scientists working on polychlorinated dibenzodioxins (PCDDs) were invited. This book records the workshop proceedings.

Research on the toxicology of the PCDDs has increased at an exponential rate since 1976. Recently published results, for example, have shown that TCDD will cause tumours in experimental animals. As the workshop proceedings contain no discussion of the potential carcinogenicity of the dibenzodioxins and make only cursory reference to mutagenicity studies, its coverage of the toxicology of these compounds is incomplete.

That section of the book dealing with the chemistry is, nevertheless, useful. Accounts are given of methods currently in use for the analysis of environmental samples containing PCDDs by various combinations of gas liquid chromatography, mass spectrometry and mass fragmentography; and there is some discussion of the prob-

lems encountered in separating dibenzodioxins from other industrial pollutants (for example, polychlorinated biphenyls).

But perhaps of most interest will be the information on decontamination methods following TCDD pollution. Both incineration and photolysis are considered. Temperatures in excess of 800 °C are necessary for the destruction of TCDD—at lower temperatures (300–500 °C) more of the dibenzodioxin is formed from precursors of TCDD and 2,4,5-trichlorophenolate.

The processes of photolysis are covered in four separate chapters. In the presence of a hydrogen donor such as methanol, ultraviolet irradiation of TCDD will selectively remove chlorine atoms from the molecule, thus rendering it less toxic. Alternative solvents/hydrogen donors are required for field conditions, and the action of two such combinations (ethyl oleate and xylene, olive oil and cyclohexanone) is described. Results reported from field trials at Séveso suggest that the olive oil/cyclohexanone method effectively promotes the degradation of TCDD, and that little of the dibenzodioxin was leached into the soil, despite heavy rain during the experiment.

Two potential decontamination methods not referred to are chlorinolysis and bacterial action. Their omission is not particularly serious; however, as bacteria are probably responsible for the detoxification of most of the TCDD in the environment these are organisms which cannot be ignored.

On the whole the book should prove useful as an introductory text for students of the dibenzodioxins.

Alastair Hay

Alastair Hay is Research Fellow in the Department of Animal Physiology and Nutrition at the University of Leeds, UK.

Large amplitude vibrations in molecules

Internal Rotation and Inversion: An Introduction to Large Amplitude Motions in Molecules. By D. G. Lister, J. N. Macdonald and N. L. Owen. Pp.246. (Academic: London and New York, 1978.) £11.50.

LARGE amplitude low frequency vibrations comprise a somewhat specialised topic within the subject of molecular vibration-rotation spectroscopy. However, they comprise a topic of particular interest for a number of reasons and are thus a deserving subject for a specialist text. The interest arises because these vibrations represent motions along the valleys in the molecular potential energy surface, so that they are of especial interest in the molecular dynamics of conformational changes and chemical reactions. Also, the experimental data on the energy levels of low frequency vibrations may be extensive (in contrast to the usual situation for other vibrations), and the theory by which they are related to the potential energy surface does not fall into the usual pattern of vibrational theory. Thus, in principle a book on this subject might be of interest to a wide range of chemical physicists.

This book starts with an introductory chapter on the concept of large amplitude vibrations, with many examples, and diagrams of sections of potential energy surfaces. Chapter 2 deals with basic theory, chapter 3 gives a cursory account of experimental methods (microwave, infrared, Raman and NMR spectroscopy, and electron diffraction), chapter 4 reviews *ab initio* and semi-empirical potential surface calculations, and the remaining five chapters discuss particular examples of large amplitude vibration in more detail—essentially internal rotation, inversion and ring puckering vibrations, and conformational motions in macromolecules.

Although the physical ideas are well presented, the authors have attempted to write a book which is not too taxing in mathematical theory and quantum mechanics. Sympathetic as I am to this ambition, the serious student has to face up to the theory, for indeed the relationship of experimental spectra to potential surface should be the unifying theme of the subject. Thus, I found the book weak in the very area where I feel it should be strong. The theory is loose where the student wants rigour; the material is badly organised; some important topics are not mentioned and others are discussed twice in different places without any ap-

parent correlation. As examples of these defects: on p23 "transpose" should read "conjugate transpose"; equation (2.45) on p42 lacks a factor $h^2/2I$; different methods of solving the Mathieu equation are discussed on p42 and p111 without any cross reference or comments to help the reader; and the effects of inversion on rotational constants are also discussed twice, on p58 and p70, again without cross reference (why is a first power term in x included on p70 and not on p58?), although one might reasonably have expected to find this discussion in chapter 7 or chapter 8. Many passages give me the impression that they will be of little help to a student who does not already know the subject, and of even less help to the student who does; as examples of this, consider the page about Van Vleck transformations

(p25–26), or the four pages about gas electron diffraction (p81–84). The important topic of the symmetry of non-rigid molecules, developed in the past ten years by Longuet-Higgins, Hogen, Bunker, and others, does not seem to be mentioned anywhere in the book. Quasi-linear molecules are an important class of molecules with large-amplitude vibration that are not discussed.

The book has its good points; one of them is indeed the subject itself! But in today's sophisticated and competitive world of specialist scientific textbooks, I feel that it has not been written with the care and attention to detail that are necessary for success.

Ian M. Mills

Ian M. Mills is Professor of Chemistry at the University of Reading, UK.

Thin films and the electronics industry

Thin Films: Interdiffusion and Reactions. Edited by J. M. Poate, K. N. Tu and J. W. Mayer. Pp.578. (Wiley: New York and Chichester, UK, 1978.) £25.

MANY reasons are advanced to explain why our scientific and inventive abilities in the physical sciences are frequently not realised in industry. One good reason is the failure by scientists and industry alike to treat product design and manufacturing processes as vital technologies which must develop their own specialised sciences if inventiveness is to be fulfilled in the market. We all know, without effect, that this common neglect stems from our history when science developed rapidly and modern industry was founded, both occurring almost independently. This unsatisfactory division rooted in the scientist of independent means and the practical manufacturer has led to the notions that the study of how one makes things and how they perform is too close to common work to be regarded as suitable activities for trained minds; and in any case such investment is wasteful. Only during the last War did scientists effectively join with industry to develop expertise; but so quickly did we slip back that we now seem to have no defence against another crisis—that of market competition.

If one doubts the validity of these comments then it is only necessary to read recent views of the decision of the National Enterprise Board to enter the microprocessor field. Expert opinion on

this relates to financial investment problems, preference for licencing overseas know-how, industrial need for microprocessors regardless of dependence on supply, and the novelty of the electronics and their social effects. Nowhere have I found any mention of what must be done to found a satisfactory industry in the UK for the manufacture of large scale integrated electronics. For example, who supplies the process plant? A new area of electronics cannot be established without the association of scientists and engineers developing production and product know-how and training skilled workers, that is, if the enterprise is to be more than a manufacturing unit with off-shore control of every aspect of its activities.

The book under review dealing with problems encountered in making and operating solid-state devices illustrates what must be done to establish such an industry. It also shows how US companies are able to create the industrial science needed for the purpose at hand. There remarks are not intended to be disparaging of the work done by R & D scientists in solid-state laboratories in the UK but to point up the effects of inadequate R&D funding in an industry which once enjoyed a position next to the US in the world league.

The book's editors have experience of the subjects treated from research in their laboratories. Dr Poate, a one-time Harwell Fellow, is now with Bell Laboratories; Dr Tu is a specialist in solid-state diffusion at the IBM Thomas J. Watson Research Center; and Professor Mayer is concerned with ion/atom collision effects at the California Institute of Technology. Their contributions are supplemented by others from research colleagues and members

of the laboratories of Sandia and Philips (Amsterdam). These authors have related the thermal, chemical and electrical effects occurring in semi-conducting devices to the materials and growth processes used in their fabrication. There are chapters on metal/metal interdiffusion, electromigration in thin films, electrode/compound substrate reactions, depth analysis and thin film deposition.

I read the chapters on atom transport and chemical reactions with interest because of their topical nature and because I was least well acquainted with their subjects. A new micro-metallurgy is introduced in which the conditions for grain boundary diffusion, intermetallic and silicide formation and electromigration in a variety of film materials is treated over depth and surface dimensions of a fraction of a micrometer. As is essential in a book of this kind the text is well illustrated with micrographs.

I did not expect a detailed description of deposition techniques but a balanced and informed account of those in common use. Thus I question the wisdom of referring to ion beam deposition as a promising new technique while omitting mention of plasma chemical procedures which seem to have an important future in silicon technology. Also the reader is not informed that ion beam deposition is only possible when the self-sputtering yield of the incident ions is below one. Further, without experimental evidence it is unsatisfactory to suggest that ion bombardment may aid epitaxy; the case cited of polycrystalline carbon is for deposition on a non-crystalline substrate. Generally, ion impact results in damage.

Sputtering is listed under collected deposition techniques as having the disadvantages of causing substrate damage and heating. Mention could have been made of sputtering apparatus with crossed electric-magnetic fields (magnetron) to deflect electrons from the substrate and reduce heating. It is an over simplification to lump together under "damage" particle and radiation effects which have different sources and intensities in sputtering plant operating from 10^{-3} to 10^{-1} torr gas pressure. The treatment of mixed component sputtering is not consistent. In the discussion of depth analysis (p149) it is said correctly that after an induction period components are released at rates proportional to their bulk contents, but on p95 it is stated that this only happens if there is no preferential sputtering to change the target surface composition. Few mixtures can have sputtering yields directly proportional to their contents but preferential sputtering raises the surface content of the less favoured

component thereby aiding the latter's emission. As the emission of a component is proportional to the product of its surface content and sputtering yield, an equilibrium is reached under which the emission ratio is equal to the component ratio of the bulk material.

It is always dangerous to refer to a reference by way of another (in this case Chopra for Gillam) as on p95 when discussing the enriched Au layer formed on AuCu₃ alloy during Ar sputtering. The enriched layer is stable and results in component emission with the bulk contents ratio and not that of the enriched layer as stated on p95.

The above criticisms are of subsidiary material and they do not detract from

the value of the main text in dealing with the behaviour of metal/semi-conductor systems. As a reference work it will be of value to those who want to obtain a comprehensive picture of operational problems in solid-state electronics and also those who are entering the field to produce electronic devices. The authors and the Electrochemical Society (who supported the work) are to be commended for recognising its need and for its useful preparation.

L. Holland

L. Holland was director of R&D at Edwards High Vacuum, and is now responsible for the Unit for Plasma Materials Processing at the University of Sussex, UK.

Asiatic ungulates

Mountain Monarchs: Wild Sheep and Goats of the Himalaya. By George B. Schaller. Pp. 425. (Chicago University Press: Chicago and London, 1978.) £17.50.

THIS book, the latest in the generally excellent Chicago University Press series on wildlife behaviour and ecology, reports the results of three years of field work with the large mammals of the Himalayas, with emphasis on the wild sheep and goats of the region. The work will undoubtedly stand as the foremost source of information on these Asiatic ungulates for many years. One short chapter describes observations of the large carnivores of the region.

The book is generally organised into chapters dealing with broad biological topics such as distribution, physical attributes, herd dynamics and courtship behaviour. In each chapter, the array of species is treated according to the level of information available. Narrative or tabular summaries of the biological attributes of each species are generally lacking. This fragmented mode of presentation makes the work less readable than other books in the same series. The organisation of the book, together with its enormous geographical scope and the unevenness of information on various species, makes reading frustrating and confusing at times. Nevertheless, the author is to be commended for putting together in one volume the results of years of field-work under incredibly difficult conditions and an exhaustive review of the relevant literature, scanty though the latter is. Fortunately, the index is reasonably complete, although it cannot be depended upon to ferret out every piece of information, nor is it very

helpful in dealing with the vast and confusing array of place-names.

The author concentrated his field-work on three species in Pakistan, Nepal and India: urial (*Ovis orientalis*), wild goat (*Capra aegagrus*) and bharal or blue sheep (*Pseudois nayaur*). The information on bharal is probably the most valuable and interesting. This little-known species possesses a complex mixture of sheep-like and goat-like behavioural and morphological characteristics. Schaller concludes (p44) that, "In general, bharal are considered aberrant goats, with sheep-like affinities". Again, however, the reader must refer to many pages of text, scattered throughout the book, to construct a cohesive picture of the biology of this species.

A concluding chapter on the evolution of the Caprinae provides a new perspective on this topic based on the author's own data on Asiatic caprids and literature review. This discussion of evolution and dispersal presents some counterpoints and alternative hypotheses to those of V. Geist (*Mountain Sheep*, Chicago University Press, 1971). Taken together, the two books provide a provocative basis for further research. However, as the author makes abundantly clear in his concluding remarks on the conservation of the Himalayan fauna, time is running out. Many species have been fragmented into small isolated populations, occupying habitats drastically altered by man. The reader is left with the clear impression that even the best system of reserves in the Himalayas will be inadequate unless broad changes in land-use practices can be instituted. In this regard, the enormity of the problems, economic, ecological and political, to be faced is evident.

Peter C. Lent

Peter C. Lent is with the US Fish and Wildlife Service, Anchorage, Alaska.